THE INHERITANCE OF PROTEIN CONTENT IN THE MILK OF DAIRY COWS.

by

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PART I.

THE INHERITANCE OF PROTEIN AND CASEIN CONTENT IN THE MILK OF DAIRY COWS.

INTRODUCTION.

It is a well established fact that milk yield is a hereditary character.

It is not logical to study the inheritance of the quality of milk as a unit because milk is a mixture of water, sugar, fat, protein, minerals and vitamins. The proportions of these show considerable variation under various sets of circumstances not the least of which is the individuality of the cow. Since butter-fat is the chief constituent in butter-making and since accurate figures for it may easily be obtained by simple analytical methods, it was the first constituent to be studied genetically.

As regards milk constituents other than butter-fat, not very much is as yet known. The majority of the investigators have studied the problem from the nutritional or environmental aspect rather than from the genetical point of view.

The Proteins of Milk.

The importance of milk protein in human nutrition cannot be over-estimated as it is one of the most important sources of nitrogen; especially for infants who cannot digest meat. Casein is the primary constituent of cheese, and it is as nutritive as meat protein weight for weight.

Although milk proteins are so important, not much work has been done on them from the genetic point of view. This is due mostly to the difficulties involved in the chemical analysis for protein and casein determinations.

The present investigation was planned to study the effect of the environmental conditions and the inheritance of milk proteins.

CHEMICAL ANALYSIS.

Aliquot portions from the milk (morning and evening samples in the case of cows milked twice daily, and 8 a.m., 4 p.m. and midnight samples in the case of cows milked thrice daily) were measured out in a cylinder. They were mixed thoroughly and after standing one minute, which was sufficient to remove air bubbles, but not long enough to allow the fat to separate, 10 ml. were pipetted from the beaker containing the mixed sample into a 150 ml. beaker and covered with a watch glass. The milk was shaken again by pouring it into a conical flask - the same one which was used for the a.m. part of the sample under examination - and pouring the milk again into the beaker. After one minute another lot of 10 ml. was pipetted into a 100 ml. covered beaker. By the same pipette used for the last two estimations 10 ml. were pipetted into a weighed weighing bottle. The weight of the milk in the weighing bottle - to the nearest (.01 g.) - was taken as the actual weight of milk in each beaker.

Precipitating the Casein.

Not less than 50 ml. of distilled water at $40^{\circ} - 42^{\circ}C$ were added to the 10 ml. of milk in the 150 ml. beakers followed by 1.5 ml. of 1.67 N. acetic acid (prepared by weighing 100 g. of glacial acetic acid and mixing it with water in 1000 ml. graduated flask and adding distilled water up to the mark). The mixture was stirred with a glass rod very gently 4 - 5 times. The beaker was left undisturbed for 20 minutes (Moir, 1931) after which 4.5 ml. of 0.25 N. sodium acetate was added, with gentle stirring, and then allowed to stand for 1 hour.

Filtration, digestion and distillation were done as described by Azarme (1938a).

Titration and calculation.

After distillation the conical flask was cooled by placing it in a basin containing cold water and then neutralised by $\frac{N}{10}$ sodium hydroxide. The percentage of casein nitrogen was then found.

Determination of the Protein percentage.

Ten ml. of 10% trichloracetic acid were added to the 10 ml. milk in the 100 ml. beaker, stirred and filtered quickly (within a few minutes) as longer contact of the acid with the milk is apt to hydrolyse the precipitated proteins. The same kind of filter paper as in the casein determination was used. The precipitate and filter paper were, without washing, transferred to a 500 ml. Pyrex Kjeldahl flask containing a catalyst. Twenty ml. of distilled water and 10-15 ml. of concentrated sulphuric acid (nitrogen free) were added to the beaker to remove the adherent parts of the precipitate, and transfer it to the flask. The washing was carried on with the rest of the 30 ml. of acid (automatic burette was used) until the beaker was clean. Washing twice was usually sufficient. The contents of the flask were digested and distilled as in the case for the casein determination.

Albumin + globulin Nitrogen calculation.

The albumin + globulin nitrogen percentage was calculated by subtracting the percentage of casein nitrogen from the percentage of total protein nitrogen.

Blank experiments were carried out (in duplicate) during the work every three weeks. They varied between .18 and .30 ml. of $\frac{N}{10}$ sulphuric acid. This amount was subtracted from the amount of sulphuric acid that was apparently neutralised by the ammonia of the proteins or casein.

Chemical Discussion.

It is known that the casein becomes completely insoluble at its isoelectric point pH 4.67. For precipitating several samples from different cows we add the same amount of acid and buffer to the same volume of milk from each sample. Since the original milks (samples) have not necessarily the same pH value, we might expect that the final solutions in which the casein has been precipitated will not have the same pH.; hence the precipitation of casein may not be strictly identical for each sample. It would be impracticable to find out for each sample the correct amount of acid to be added to reach the isoelectric point of casein in the final solution, and it is extremely unlikely that the discrepancies would be of importance.

Davies (1933) following Moir's method found that the casein nitrogen more or less uniformly accounted for 76% of the total nitrogen in the normal samples and for 80% of the protein nitrogen. Rowland & Zein-El-Dine (1938) found that the casein (determined by Rowland's method ; 1938a) in the milk of healthy cows accounted for at least 78% of the total nitrogen. The difference in the two results may be attributed to the inclusion of some samples from unhealthy udders, in Davies' data. This can be detected from inspecting the casein number of his results: cows giving milk with $\frac{\text{N.P.N.}}{\text{T.N.}} = .15$ are sure to have very low casein numbers and are sure to be mastitis cows.

Mattick & Hallett (1929) for determining the casein nitrogen in samples from individual quarters of a cow used, "10 ml. of milk and 90 ml. of water at 42° - 43°; the casein was precipitated by 1.5 ml. of 10% solution of acetic acid and filtered". The method used by these workers is open to doubt in the light of Moir's work (1931) who showed that addition of a buffer after the acid is essential for the complete precipitation of casein.

Cranfield et al (1927) and also Gaines & Overman (1938) calculated the "Total protein" from total nitrogen X 6.38. It would have been better to refer to the "nitrogen X 6.38" as the nitrogenous part or fraction of the milk since it is not only protein but contains about 5 - 6% of non protein nitrogen (Rowland 1938b, M. Zein-El-Dine, 1939).

Macdowal & Macdowell (1936a) found that the casein content of milk can be determined with reasonable accuracy by formol titration of phenolphthalein of the curd obtained on precipitating 20 ml. with acetic acid and sodium acetate. The formol titre X 1.05 gives the casein content in grams per 100 g. of milk. The authors, however, mention that such a method is approximate and recommended only for cheese-making factories.

They found (1936b), when using Moir's method for precipitating casein, that by adopting 5 and 15 minutes as intervals before adding sodium acetate and before filtering respectively, neither the final pH nor the casein result was affected. However, recent work is not in favour of such short intervals (Rowland 1938a).

Determining the standard errors involved in the chemical work.

The majority of the present determinations were single tests, with duplicate observations in the few doubtful cases. The standard errors for protein, casein and albumin + globulin were carefully determined by the following method: A mixed sample representing the 24 hours milk yield of an Ayrshire cow during her 5th month in milk was used. Eleven determinations for protein and eleven for casein were carried out. Eleven weighing bottles each of them containing 10 ml. were weighed and the net weight of milk was found. This can be used to check the magnitude of the error involved in pipetting out the 10 ml. milk, which was taken for the protein and casein determinations respectively.

The results are shown in Table I. In previous work by others the standard error was found to be bigger (Azarme, 1938a).

TABLE I.

EXPERIMENTAL ERROR IN DETERMINATION OF NITROGEN.

Weight of 10 ml. sample	T.P.N. in 10 ml.	% T.P.N.	C.N. in 10 ml.	% C.N.	% A. + G. N.
10.24	.05369	.524	.04458	.435	.089
10.25	.05347	.522	.04458	.435	.087
10.24	.05377	.525	.04413	.431	.094
10.24	.05386	.526	.04452	.435	.091
10.25	.05352	.522	.04446	.434	.088
10.24	.05236	.512	.04452	.435	.077
10.25	.05341	.521	.04458	.435	.086
10.24	.05380	.525	.04441	.434	.091
10.24	.05397	.527	.04387	.429	.098
10.25	.05369	.524	.04474	.437	.087
10.24	.05369	.524	.04474	.437	.087

	Mean	Standard error
Percentage of T.P.N.	0.523	±0.0013
" " C.N.	0.434	±0.0008
" " A. + G.N.	0.089	±0.0017

Comparing two methods for casein precipitation.

Several methods have been employed for casein determinations. The best one is generally accepted to be the method of the A.O.A.C. with the improvements introduced by Moir (1931). The analysis of this investigation had been carried out with Moir's method for several months, before Rowland (1938a) published his new technique in precipitating casein. He states that the figures obtained by his method are 2.4 and 1.0% higher than those given by the A.O.A.C. and Moir's methods respectively.

The two methods were compared. The technique used in precipitation, digestion and distillation is mentioned above (page 2). All the results are mean of duplicates which agreed closely. The correction factor in Rowland's method for casein was taken to be .995.

Ten samples chosen from cows of different breeds at different stages of the lactation were used to determine the better method of the two.

Results obtained are shown in Table II.

TABLE II.

SHOWING NITROGEN FIGURES OBTAINED BY DIFFERENT METHODS.

Name of Cow	Rowland's Casein (by difference)	Rowland's Casein X.995	Moir's Casein	Difference R. - M.	% Difference
	g. in 100 ml.	g. in 100 ml.	g. in 100 ml.		
1. S.D.L.	.4697	.4652	.4714	-.0062	-1.32
2. Manique	.3767	.3748	.3694	+.0054	+1.46
3. B.O. Blossom	.3865	.3847	.3792	+.0055	+1.4
4. C. Dapple	.4070	.4049	.4081	-.0032	- .78
5. S.R. Prin.	.4150	.4129	.4107	+.0029	+ .54
6. R.'s Rosebud	.3995	.3974	.4003	-.0029	- .72
7. B. Jess	.3895	.3875	.3805	+.0070	+1.84
8. B. Nessie	.3890	.3871	.3892	-.0021	- .54
9. S.R. Clipper	.4806	.4781	.4807	-.0026	- .54
10. C. Daffodil	.3377	.3360	.3310	+.0050	+1.51
					-3.9 +6.75
				Total	+2.85
				Mean	+0.285

It may be noted that the milk of 3 out of 4 shorthorn cows gave higher results by using Moir's method, while 4 out of 5 Ayrshires behaved in the opposite way.

The result shows that of ten cases five were negative. The mean percentage increase in the amount of casein when using Rowland's method was found to be +0.285% (S.E. ±0.38) in this work. If the factor .992 was applied at least for cows 1, 5 and 9 (being high casein and fat yielders) the difference is sure to be less than that.

We cannot consider the difference between the two methods as significant since it is less than its standard error.

MATERIAL USED.

The cows were selected by breed and calving date from the experimental farms of the Institute of Animal Genetics at Shothead and Cockburn. They were mostly Ayrshires but included Shorthorns, Jerseys, crosses of these breeds and one cross Ayrshire/ Guernsey. Full particulars of each cow are to be found in Appendix I. The number of cows tested was 105, but unfortunately, owing to death and disease, only 89 are included in this study.

Health of Cows.

Cows are closely observed at the farm. No cow under the investigation has aborted. The herds are attested and the tuberculin test was carried out every 6 months, any cows showing positive results being disposed of. Any cow showing symptoms of being infected with mastitis (whether by observation on the farm, or on the basis of chemical or bacteriological tests) was excluded from the present work. One case of acetonùria occurred. It can be said with confidence that every cow that was included in the statistical analysis was healthy from every relevant respect.

Feeding of Cows.

The cows are kept under standard rations.

Concentrates are given to the cows according to their milk yield. The following is the ration for in-milk cows:-

maintenance ration:-

25 lbs. turnip

10 lbs. Hay

5 lbs. straw (or hay)

2 lbs. maize

Straw was omitted when the daily yield reached or exceeded 4 gallons.

production ration:-

3 parts oats

1 part bran

½ part soya bean meal

¼ part decorticated earthnut cake

¼ part ordinary bean meal

1 part maxtrex

is fed at the rate of 4 lbs. per gallon.

Other Points in Managing the Herd.

Milking is done by machine and is carried out twice daily at 11 a.m. and 10 p.m. Heavy milkers are milked at 8 hours intervals; the first milking is started at 8 a.m.

Intervals between sampling.

To arrive at the true figure denoting the actual amount of proteins secreted by individual cows during the whole lactation, it is necessary to analyse daily the milk of each cow under investigation, which is obviously impracticable.

Azarme (1938b) had already determined the protein in the milk of cows at intervals of seven days. Using 27 cows he arrived at the conclusion that taking weekly samples as a basis of comparison, the fortnightly samples involve errors which will be found within the limits of ± 5.84; while for three-weekly samples the errors will fall within the limits of ± 5.98.

He also showed that 3 weekly sampling is accurate enough for the latter part while 2 weekly samples were desirable for the first part of lactation.

In this study, to begin with, the analysis was carried out weekly; but in order to obtain the protein yield for as many cows as possible without increasing the labour, subsequent determinations were carried out every 2 weeks during the first part of lactation (3 - 5 months) when the cow is giving a high milk yield, and every 3 weeks thereafter.

ENVIRONMENTAL FACTORS AFFECTING PROTEIN SECRETION.

It has been shown that total milk yield and also yield of fat are affected by many environmental factors as breed, age, length of lactation, persistency, month of calving, the dry period preceding the lactation as well as by others, some of which cannot be measured accurately. In a study of the genetic aspect, we must take into consideration the effect of the environmental conditions in order to arrive at a good estimate of the hereditary influence.

From preliminary studies on the total protein yield of cows four factors appeared to have influenced it. It was decided then, to correct the "raw" protein yield for month of calving, age of cow and service period.

(a) Age of Cow.

It is generally agreed that milk yield tends to increase with age up to around 6-9 years of age when maximum yield is reached.

Tocher (1925) found that the percentage of lactose, casein and solids-not-fat, as well as the

TABLE III.

Amounts of protein and casein (in lbs.) secreted by different cows during lactation; and the protein and casein yields after correction for age, month of calving and service period.

No.	Name.	Amount of protein secreted in lb.	Amount of Protein after correction	Amount of casein secreted.	Amount of Casein after correction.
1.	C.Ada	341.5	397	286	333
2.	B.Jess	410	398	333.7	295
3.	B.O.Blossom	541.3	470	384	334
4.	C.Bogie	270.3	307	222.6	253
5.	C.Calmette	85.2	104	68.5	83
6.	C.Celandine	235.7	279	177.4	207
7.	C.Constance	261.5	310	217.3	258
8.	C.Cora	207.4	198	168.7	195
9.	C.Countess	238.2	279	187.7	220
10.	C.Daffodil	285.5	297	239.8	288
11.	C.Dosie	218.3	255	180.8	211
12.	C.Fanny	264.7	331	225.8	283
13.	C.Fidget	298.5	349	250.8	293
14.	C.Gladys	366.6	413	298.5	336
15.	C.Hilda	253.2	311	211.3	260
16.	C.Jess	379.5	399	315.6	332
17.	C.Madge	257.2	293	214.8	244
18.	C.Marjorie	208.4	259	161.8	201
19.	C.Missie	268.4	285	216	230
20.	C.Nequita	306.5	327	250.2	267
21.	C.Nessie	219.4	257	185	216
22.	C.Y.Kate	361.2	302	297.6	248
23.	D.Mona	316.5	294	245.3	228
24.	E.Rosebud	153.7	146	121.3	116
25.	L.N.G.Lura	110.5	96	87.3	76
26.	L.M.Queen 4th	209.0	227	171.6	185
27.	L.B.Polly 14th	318.4	350	263.5	290
28.	L.Crummie	241.7	245	198	201
29.	L.Dandy	311.2	307	255.3	252
30.	Manique	192.8	234	156.2	189

TABLE III. (Cont'd.).

No.	Name.	Amount of protein secreted in lb.	Amount of Protein after correction	Amount of casein secreted	Amount of Casein after correction.
31.	W.Vanity	278.2	330	232.5	276
32.	S.Alannah	195.9	215	158.4	161
33.	S.Blossom	110.7	110	92	91
34.	S.Camilla	268.0	212	204.6	162
35.	S.Ethyl	398.6	430	321.3	323
36.	S.Gretchen	290.7	276	236	225
37.	C.Charlotte	255.6	260	218	222
38.	Pr.Bina	339.2	345	276.7	282
39.	C.Florence	326.3	382	264.3	309
40.	Hecuba	149.4	178	123.8	148
41.	S.Campanula	268.8	306	215.6	245
42.	A.Bloomer	321.2	389	255.8	308
43.	L. of Foot's Fairy	82.8	91	67.6	74
44.	C.Jeanette	275.7	336	194.5	237
45.	C.Bundy	died	–	–	–
46.	L.Hilda	248.0	314	198	251
47.	S.Patricia	92.5	122	77.4	102
48.	A.Buntie 66th	346.2	376	289.5	314
49.	A.Dandy 21st	243.8	265	193.4	210
50.	M.Aster	296.9	322	244.7	266
51.	Clockgirl	reacted T.T.	–	–	–
52.	P.W. Perf.5th	257.8	329	206.7	264
53.	C.Roberta	131.4	192	110.2	161
54.	P.Bernice	231.4	295	187	239
55.	B.Magpie 2nd	362	419	307	356
56.	Patsy	177.9	245	147.8	204
57.	S.Adeline	174.7	254	146	212
58.	S.P.Lustre	64.1	80.0	49.2	61
59.	S.Greta	254.0	354	207.5	289
60.	Caldaria	108.2	124	88.8	102

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TABLE III. (Cont'd.).

No.	Name.	Amount of protein secreted in lbs.	Amount of Protein after correction	Amount of casein secreted.	Amount of Casein after correction.
61.	Ca.Dewdrop	235.3	214	183	166
62.	C's Cowslip	241.8	357	198	292
63.	L.Keepsake	254.8	333	209.7	274
64.	Meg Ramsay	147.9	231	119	186
65.	M.Ethel	177.6	201	138.4	157
66.	S.Pink	72.4	107	58.5	86
67.	S.Sharon	97.8	115	75.5	89
68.	B.Countess	408.9	411	336.2	338
69.	P.Album	291.8	384	240.2	316
70.	P.W.St.3rd	318.0	351	253.7	280
71.	A.Y.Kate	254.4	249	201.7	197
72.	Ca.Pink	210.0	268	162	206
73.	C's Sharon	199.1	278	164.3	229
74.	S.Harriet	255.8	360	213	283
75.	S.Calyx	151.4	214	124.4	175
76.	S.Henny	167.7	230	140.2	193
77.	S.L.Lap	203.4	330	167.2	271
78.	S.P.Beauty	89.9	109	72.6	88
79.	S.Caducia	257.0	304	200.6	237
80.	A.Ada	314.5	338	267	286
81.	L.F.Beauty	322.4	325	268.8	271
82.	A.M.Craig 67	283.6	316	223.7	254
83.	S.Capitvation	reacted T.T.	–	–	–
84.	S.R.Clipper	139.7	197	114	161
85.	S.Shirley	312.4	399	258	330
86.	L.M.Queen 3rd	275.5	281	215	220
87.	S.Blonde	134.8	190	111.5	157
88.	S.Telluria	168.3	233	135.2	187
89.	B.Nessie	427.0	399	352.5	328
90.	C.Mistress	187.4	237	156.3	198
91.	S.D.Lustre	198.4	205	163	168
92.	C.Dapple	157.5	195	128.6	159
93.	C.Yvette	137.2	156	111.5	127

refractive index and specific gravity fall with age. On the other hand, the percentage of albumin rises gradually to 10 years of age.

He also found that age has an effect on the butter-fat percentage ($r = -0.1808 \pm .035$); this means that the percentage of butter-fat tends to decrease with age.

With regard to the relationship between solids-not-fat and age, he found that there is a uniform fall from 8.9 per cent at three years of age to 8.6 per cent at 13 years, while Bartlett (1934a) demonstrates a decrease of .5 per cent solids-not-fat between the first and the ninth lactation period.

Tocher used individual samples from cows from different farms of Scotland. In the present study the total protein yield for each cow was used. Cows were grouped into classes. The average protein yield for each class was found by dividing the summation of the total protein yields by the number of cows in the same class. After finding the actual values of the classes the theoretical value was found by using the "Method of least squares". The difficulty was that not all the classes were of the same weight, i.e. the number of cows in each class is not equal to the number of cows in the other classes. Owing to the fact that the number of cows included in the study is not many (89 cows), some classes showed great deviation from the general trend. This can be attributed to randomisation. Such points were taken into consideration while fitting the curvé. Some writers take the age of cows in lactations but this is not fair for many cows. In the present study two cows began their first lactation at 25 and 62 months respectively. Such a cow as the second must be given a handicap.

If we call the periods 24-29, 30-35 (see Table IV) $t = 0, 1, 2, 3 - - - - 11, 12, 13,$ and if we put provisional values for the very doubtful data with small frequencies at $t = 0, 5, 7, 13$ and fit a straight line by least squares we get the regression.

$y = 207.82 + 9.69\, t$ ....(1)

which gives the figures shown in Table IV. If we fit a parabola we get:-

$y = 204.17 + 11.72t - 0.168\, t^2$.......(2)

giving theoretical values as shown in Table IV.

Because protein yield is highly correlated with milk yield and with yield of butter-fat, it is not supposed that the increase with age will be indefinite, so the values given by the parabola were taken to calculate the correction factors.

If we determine the goodness of fit of the theoretical and actual values, we get the result shown in Table (4A). This indicates that the fitting is satisfactory since the deviation of the theoretical from the actual value is less than the standard error of the latter.

Graph I shows the effect of age on the yield of total protein.

TABLE 4.

Showing the mean protein yield in lb. for each group of cows of same age.

Age.	No. of cows.	Mean amount of protein secreted.	Theoretical values	
			Straight line	Parabola
24-29	1	85.2	208	204
30-35	20	222.7	218	216
36-41	8	216.5	227	227
42-47	10	227.8	237	238
48-53	13	253.2	247	248
54-59	3 (2)	161.2 (250.4)	256	259
60-65	8	255.	266	269
66-71	2	93.9	276	278
72-77	4	268.7	285	287
78-83	4	344.6	295	296
84-89	6	318.7	305	305
90-95	1	427.	314	313
96-101	4	295.5	324	321
Over 102 (108)	1	177.6	334	328

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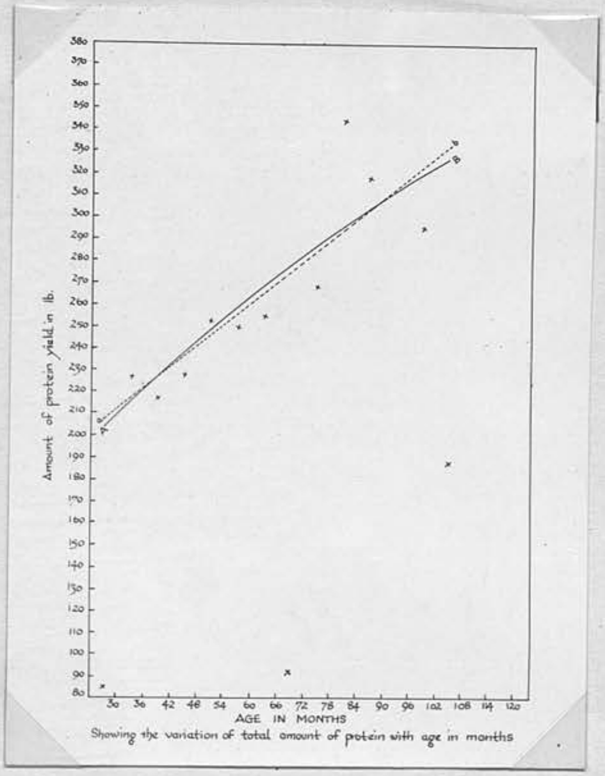
TABLE 4 A.

Showing details of the test of goodness of fit of the actual and theoretical values for the mean protein yields secreted by cows of different age (only groups of 4 cows or more are tested).

Age (in months)	Difference between theoretical and actual values (parabola).	Standard Error of the Mean.	Difference between theoretical and actual values (straight line).
30-35	- - 6.7	15.11	- 4.7
36-41	+ 10.5	15.11	+10.5
42-47	+ 10.2	31.45	+ 9.2
48-53	- - 5.2	13.48	- 6.2
60-65	+ 14	30.5	+ 11
72-77	+ 18.3	22.99	+ 16.3
78-83	+ 48.6	90.6	+ 49.6
84-89	+ 13.7	18.7	+ 13.7
96-101	± 25.5	38.94	- 28.5



GRAPH I.

Showing the effect of age on the amount of protein secreted during the lactation.

(b) Month of Calving.

Sanders (1927) found that the time of calving has a great effect on the milk yield. English cows calving in October and November give higher yields than those calving in May. This confirms the practical farmer's belief. The cause for the variation may be nutritional.

Ghoneim (1936), experimenting on the efficiency of green clover in producing milk near Cairo found that the high milk yield given by dairy animals, i.e. cows and buffaloes, during the winter months, is not due to the clover itself but to the great amounts of food constituents that the animal gets owing to the cheapness of green fodders at that season. He found also that if the animals were given the same amount of starch and protein equivalents at other seasons their milk yields will not vary significantly from the yields in the winter months.

In the present study cows were grouped according to the month of calving and the average protein yield for each month was found as previously described.

Since, presumably, the curve showing the effect of month of calving on the protein yield repeats itself year after year (according to the amount of green fodder available during each month), it is presumably representable by means of a periodic function. The typical periodic function over a period comprising twelve intervals is of the form:-

$$y = a_0 + a_1 \cos\theta + a_2 \cos 2\theta + b_1 \sin\theta + b_2 \sin 2\theta +$$

where θ takes the values 0°, 30°, 60° ... 300°, 330°.

It is true that the present data are of different weight, had they been of the <u>same</u> weight the following curve would have been a good fit:-

$$y = 242.5 + 20\cos\theta - 1.25\cos 2\theta + 12.5\sin\theta + 7.5\sin 2\theta \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

when we give θ the values 0°, 30°, 60°....330°, the values of y proves to be as shown in Table 5.

The goodness of fit of this curve, when tested, was found satisfactory. Details are shown in Table (5A).

Graph II shows the yield of protein against month of calving, and Table 5 shows the theoretical values for each month. It must be mentioned that the effect of month of calving is more or less local and it is incorrect to use the correction factors

TABLE 5.

Showing mean protein yield for cows calving in different months.

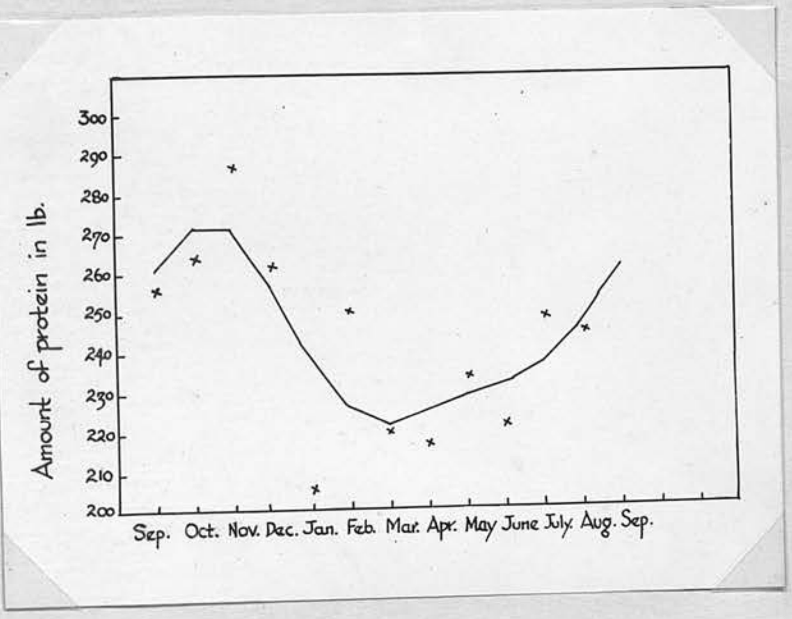
Month	No. of cows.	Mean lb. protein secreted.	Theoretical values.
Sept.	8	256.3	261
Oct.	19	264.0	272
Nov.	8	286.9	271
Dec.	4	261.7	257
Jan.	4	205.6	238
Feb.	5	251.0	226
Mar.	11	220.2	222
Apr.	13	216.5	225
May	8	234.0	229
June	2	221.9	232
July	3	249.4	237
August	-	(245)	247

85

TABLE 5A.

Showing details of the test of goodness of fit of the actual and theoretical values for the mean protein yields secreted by cows calving in different months.

Month.	Difference between theoretical and actual values.	Standard Error of the mean.
September	- 4.7	27.53
October	-8	23.99
November	+15.9	19.11
December	+ 4.7	53.95
January	-32.4	54.4
February	+25	43
March	- 1.8	22.44
April	- 8.5	24.49
May	+ 5	32.67



GRAPH 2.

Showing the effect of month of calving on the protein lactation yield.

found in one study for another one because the time of green fodder varies considerably from place to place.

(c) Service period and pregnancy.

Gaines and Davidson (1926) found that until the fifth month, the influence of pregnancy on lactation is practically negligible. Cows in the tenth month of lactation which have been eight months in calf will give possibly 20 per cent less milk than farrow (open) cows.

A high positive correlation between service period and the length of the lactation was found by Sanders (1927). He showed that the longer the service period (i.e., number of days between calving and the first fertile service) the higher the milk yield will be. He found also that the milk yield tends to settle down at a little lower level after pregnancy and after 20 weeks from the fertile service the yield decreases rather rapidly.

Bartlett (1934a) found that the rise in the percentage of solids-not-fat, which occurs during the latter half of the lactation period is associated with the subsequent pregnancy of the cow, the percentage of solids-not-fat begins to increase slightly at about half-term of pregnancy and the increase continues throughout the remainder of the lactation period. On the other hand, barren cows tend to yield milk in which the percentage of solids-not-fat decreases throughout the lactation period.

Espe (1938) states that gestation in itself does not influence the composition of milk, but it may indirectly have some effect by causing the cow to go dry. Quoting Eckles he says that on a dry matter basis a Jersey calf at birth and the accompanying fluid and the membranes are equal to 110-170 lb. of Jersey milk in energy value. In other words a cow carrying a calf over 200 days of any lactation produces 3 per cent less milk than if she has carried no calf during this period.

In the present study service period was found to have a definite effect on the protein yield as that it has on the total yield of milk, i.e. the longer the service period, the higher the protein amount secreted. In case of cows that did not become pregnant while in milk, the date of service was taken to be the date of the end of their lactation. Such cows were not included while calculating the correction factors for service period.

Cows were grouped according to their service periods and the theoretical value for each class was found by method of least squares.

Graph 3 shows that the amount of protein secreted during lactation increases with the length of service period up to the sixth period and declines after that. This does not agree with the previous writers. Most probably it is due to the fewer numbers of cows from which the last two figures were derived. To avoid committing a mistake by using the last two figures, Sanders correction factors for service period in respect of lactation yield of milk were adopted. This will not be far from the truth since the first six figures here are in agreement with those of Sanders, and milk yield shows a high positive correlation with the yield of protein. Using correction factors for the service period in one study for the other is not objectionable because the effect is purely physiological and does not vary by the change of place.

TABLE VI.

Showing mean protein yield of cows with different service periods.

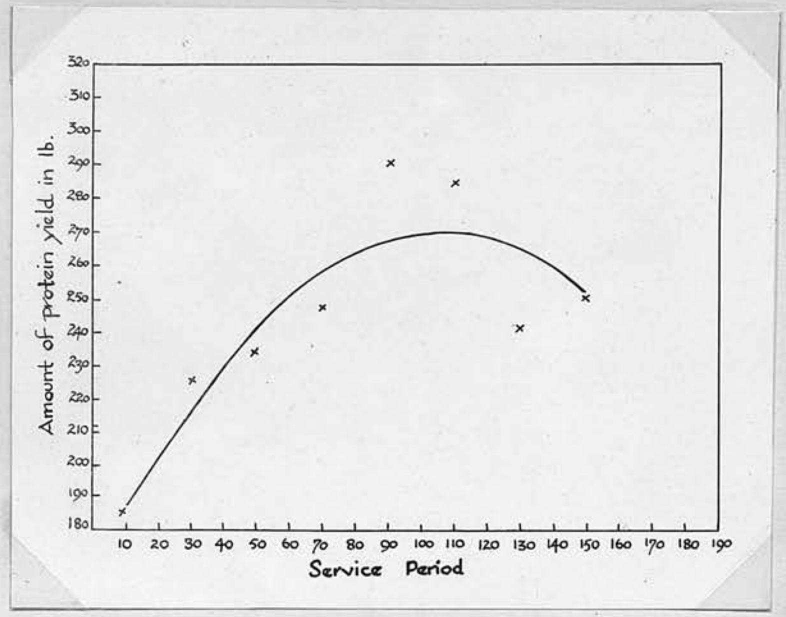
Service period.	No. of cows.	Mean protein yield in lb. (secreted).	Theoretical value
40-59	2	185.9	187
60-79	14	226.4	218
80-99	17	234.4	242
100-119	14	248.2	259
120-139	6	290.5	268
140-159	9	284.5	270
160-179	4	242.5	266
180-199 or over	4	250.6	254

70√

TABLE VI. A.

Showing details of the test of goodness of fit of the actual and theoretical values for the mean protein yields secreted by cows having different service periods.

Period	Difference between theoretical and actual values.	Standard Error of the mean.
60-79	+ 8.4	19.96
80-99	- 7.6	22.57
100-119	-10.8	18.7
120-139	+22.5	37.86
140-159	+14.5	14.49
160-179	-23.5	40.84
180-199	- 3.4	50.85



GRAPH 3.

Showing the effect of the length of the service period on the protein lactation yield.

The goodness of fit of this curve, when tested, was found satisfactory. Details may be found in Table (VIA).

(d) <u>Dry period preceding the lactation</u>.

The parenchymatous (secreting) tissue of the udder, after being active for several months, becomes "tired". It needs some rest before the beginning of the next lactation. In practice, farmers tend to keep the first-calf heifers in milk for the longest possible period. They believe that the longer such cows will be in milk, the better will be their milk yield during the next lactations.

Sanders (1927) has suggested that over-feeding of the cow during the closing stages of the preceding lactation will counteract the bad effect of a short dry period. This, however, might not hold true if we consider that in that case the drying off of the cow will be more difficult.

Espe (1938) quoting Woodward et al. (1933) says that cows would usually be dry four to six weeks before calving, depending on their condition when dried up. During this period the body reserves, especially mineral reserves, can be built up in anticipation of the severe drain which heavy production entails.

Loertscher (1937) states that the dry period should never be less than 3 weeks, while to obtain a maximum milk-yield in the subsequent lactation, it should be 8-10 weeks. He noted that when the dry period is of greater length, the subsequent yield is usually adversely affected due probably, he says, to degeneration of the tissue of the udder.

Table VII gives the yields of protein in relation to the dry period. Unfortunately the small number of cows with a short dry period does not provide evidence to show that a short period affects the subsequent lactation. There is, however, evidence in support of Loertscher's findings as to dry period of abnormal length. If the dry period exceeds fourteen weeks the subsequent lactation yield of protein is adversely affected. It must be remembered that the cows tested included some abnormally low yielders who also lacked persistency. It is the lactations of such cows that have contributed to the lower yields in respect of extended dry periods.

In view of these uncertainties no correction has been made in respect of dry period except in the case of one cow Muncraig Ethel whose dry period was only 14 days. As protein yield is highly correlated to milk yield, this particular lactation has been corrected by adding 8% to her yield, this being the figure given by Sanders (1927) for the correction of total yield of milk for a dry period of under 20 days.

TABLE 7.

Showing mean protein yield of cows with different dry periods before the present lactation.

Dry Period	No. of cows.	Mean protein yield in lbs.	Theoretical value.
0-19	1	177.9	309
20-39	2	290.9	309
40-59	7	352.9	306
60-79	12	297.7	302
80-99	8	304.1	295
100-119	6	246.7	286
120-139	3 (2)	218.4 (285.7)	275
140-159	3	243	261
160-179	3	276.3	246
180-199	2	103	228
200-219	4	187.7	208
220 and over	4	203.9	186

If we call the periods 0-19, 20-39, 40-59...etc. $t = 0, 1, 2, 3$ ....10, 11, we get the parabola

$$y = 309.21 + 0.687t - 1.078t^2$$

by least squares.

If we put $t = 0, 1, 2$.........10, 11 in this equation we get the theoretical values stated above.

Calculating the correction factors.

(1) Age.

The age of maximum production was taken to be 103-108 months. It might not be exactly valid but it is not expected to be far from the truth, since the age at which the cow gives the highest milk yield is nearly 8 years and that at which it gives the highest butter-fat yield is 7-9 years (Gowen 1924). The percentage which is needed to be added to a cow's yield at any age in order to obtain its probable yield at age of maximum production was found by subtracting the theoretical yield at that age from the theoretical yield at the age of maximum production and dividing the difference by the latter figure.

(2) Month of Calving.

The correction factors were calculated by taking the mean of the theoretical values for each month. The correction factors were arrived at by subtracting the theoretical value from the mean and dividing the result by the mean.

Service Period

(3) In the case of service period the same method as above (2) was pursued.

Correction factors for age, month of calving and service period are stated below.

see p 45 for para re pp 45-47
(Sanders tables)

TABLE 8.

CORRECTION FACTORS.

(1) Age.

Age	Correction factor %
24-29	+ 37.82
30-35	+ 34.17
36-41	+ 30.8
42-47	+ 27.43
48-53	+ 24.4
54-59	+ 21.5
60-65	+ 18.0
66-71	+ 15.25
72-77	+ 12.5
78-83	+ 9.75
84-89	+ 7.01
90-95	+ 4.57
96-102	+ 2.12
103-108	-

TABLE 9.

(2) Month of calving.

Month of calving.	Correction factor %
Jan.	+ 2.06
Feb.	+ 7.0
March	+ 8.64
April	+ 7.41
May	+ 5.76
June	+ 4.57
July	+ 2.88
August	– 1.65
Sept.	– 7.41
Oct.	– 11.94
Nov.	– 11.54
Dec.	– 5.76

TABLE 10.

(3) Service period

S.P. in days.	Correction factor %
40-59	+ 23.84
60-79	+ 11.2
80-99	+ 1.43
100-119	- 5.5
120-139	- 9.17
140-159	- 9.98
160-179	- 7.7 *
180-199 or over	- 3.46*

* These two figures are derived from very few data and so they are undependable.

AGE OF THE COW.

Standard - Maturity = 6th lactation.

Age	Correction %
1st lactation	+30.6
2nd "	+18.0
3rd "	+ 9.3
4th "	+ 3.7
5th "	+ 0.7
6th "	-
7th "	+ 1.4
8th "	+ 4.8
9th "	+10.4
10th "	+18.5
11th "	+29.4
12th "	+43.7

Sanders' correction factors for milk yield are stated on pages 45, 46 & 47 for comparison.

MONTH OF CALVING.

Standard - Mean of all months.

Month of Calving	Correction %
January	- 0.9
February	- 2.8
March	- 0.2
April	- 2.2
May	+ 3.4
June	+ 7.0
July	+ 5.0
August	+ 3.0
September	+ 0.5
October	- 4.7
November	- 2.6
December	- 3.8

LENGTH OF SERVICE PERIOD.

Standard - S.P. = 85 days.

S.P. in days	Correction 1st calvers %	Correction Others %
0-19	+ 28.2	+ 33.9
20-39	+ 18.4	+ 21.3
40-59	+ 10.6	+ 11.9
60-79	+ 4.2	+ 4.6
80-99	- 1.1	- 1.1
100-119	- 5.5	- 5.9
120-139	- 9.2	- 9.7
140-159	- 12.4	- 12.9
160-179	- 15.2	- 15.5
180-199	- 17.6	- 17.8
200-219	- 19.7	- 19.7
220-239	- 21.5	- 21.4
240-259	- 23.1	- 22.8
260-279	- 24.5	- 24.1
280-299	- 25.8	- 25.1
300-319	- 27.0	- 26.1
320-339	- 28.0	- 26.9
340-359	- 28.9	- 27.6
360-379	- 29.8	- 28.3
380-399	- 30.5	- 28.8
400-419	- 31.2	- 29.3
420-439	- 31.8	- 29.8
440-459	- 32.4	- 30.2
460-479	- 32.9	- 30.5
480-499	- 33.3	- 30.8

GENETIC ANALYSIS.

It is known that there are significant differences between different breeds in respect of amount and percentage of butterfat in the lactation. The normal percentage of fat in milk of some breeds is as much as $1\frac{1}{2}$% higher than that of others; yet the difference in test between animals of the same breed is frequently even greater. Turner and Haskell (1929) found that the size of fat globules also varies between breeds, and that there is a slight tendency for the higher testing cows of each breed to secrete milk with larger fat globules than those giving milk poor in butterfat. Edwards (1936) found that among the best representatives of the various dairy breeds there is little difference in gross efficiency of milk production.

Breed Differences.

Before tackling the problem of inheritance of protein and casein yields a study was made of the differences between the mean yields of different breeds. If there exists a significant difference this would suggest the probability of the yields of protein and casein being inherited.

In the present investigation three breeds were included - Ayrshire, Shorthorn, Jersey and also crosses between Shorthorn and Ayrshire. One cross Ayrshire/Guernsey cow was also included. The mean protein and casein yields of each group were found by dividing the summation of the corrected yields by the number of cows of the corresponding breed. The results are shown in Table XI. It must be mentioned that the protein and casein figures used are corrected for age, month of calving and service period.

TABLE XI.

TABLE XI.

Showing the mean protein and casein yields for different breeds.

Breed	No of cows.	Mean protein yield in lb.	Mean casein yield in lb.
Ayrshire	69	296.6 (S.D.75.9)	242.6 (S.D. 62.81)
Cross (Ayrshire & Shorthorn)	4	303.3 (S.D.47.3)	249.8 (S.D. 40.4)
Shorthorn	5	195.0 (S.D.50.60)	158.0 (S.D. 39.6)
Jersey	5	129.8 (S.D.59.6)	104.8 (S.D. 46.41)
(Ayrshire & Guernsey	1	178	148

When the differences between the mean protein yields of the different breeds were tested by "t" test (taking P = .05) the following results were obtained.

TABLE XII.

Showing the results of testing the significance of the differences between the means of protein yields. of different breeds by "t" test.

Breeds	Protein yields	Degrees of freedom	Value of t observed.	Theoretical value of t (Fisher's)	Result
A (Ayrshire (Jersey	297 130	68 4	4.8	1.95996	Significant
B (Ayrshire (Shorthorn	297 195	68 4	2.96	1.95996	Significant
C (Ayrshire (Cross(S.x A)	297 303	68 3	.1576	1.95996	Not significant
D (Jersey (Shorthorn	130 195	4 4	* 1.86	2.306	Not significant
E Jersey Cross (Short x Ayr.)	130 303	4 3	4.5	2.365	Significant
F (Shorthorn (Crosses	195 303	4 3	3.12	2.365	Significant

* This difference will be significant if we take the value of P = .1

From Table XII we may conclude that a significant difference exists between the mean protein yields of different breeds. The "not significant" difference between the protein yield of the Ayrshires and that of the Shorthorn x Ayrshire crosses will be discussed later.

If the significance of the differences between the mean casein yields of the different breeds is tested the following results are obtained.

TABLE XIII.

TABLE XIII.

Showing the results of testing the significance of the differences between the means of corrected casein yields of different breeds by t test.

	Breed	Casein yield	Degrees of freedom	Value of t observed	Theoretical value of t (Fisher's)	Result
A	(Ayrshire (Jersey	243 105	68 4	4.8	1.95996	Significant
B	(Ayrshire (Shorthorn	243 158	68 4	2.96	1.95996	Significant
C	(Ayrshire (Cross (S. x A.)	243 250	68 3	.223	1.95996	Not significant
D	(Jersey (Shorthorn	105 158	4 4	* 1.91	2.306	Not significant
E	(Jersey (Cross (S. x A.)	105 250	4 3	4.63	2.365	Significant
F	(Shorthorn (Cross	158 250	4 3	3.59	2.365	Significant

* Significant if we take value of P = .1 instead of .05

From Table XIII it may be concluded that there is also a significant difference between the breeds in respect of yield of casein.

If comparison of the difference between the percentages of protein in the milk of different breeds is made, the following result is obtained:-

TABLE XIV.

Showing the mean protein percentages for different breeds.

Breed	No. of Cows.	Mean Protein %	S.D.	95% probability range
Ayrshire	69	3.08	.21	2.66 - 3.50
Cross (Ayr. and Short.)	4	3.11	.16	2.79 - 3.43
Shorthorn	5	3.23	.21	2.81 - 3.65
Jersey	5	3.36	.11	3.14 - 3.58

On applying "t" test to the figures no significant difference is found between the means.

It may be concluded that there is no substantial difference between breeds in respect of the percentage of protein in milk, although a significant difference in the percentage of butterfat has been observed by others.

Effect of Crossing two breeds on the protein and casein yields of the first generation (F_1).

In the present investigation the protein and casein yields of four cows (three of which are by Shorthorn bull x Ayrshire cow and one is by Ayrshire bull x Shorthorn cow) were obtained. Looking at Tables XI, XII and XIII, we find that the mean protein and casein yields of these four cows (being the first generation) is very near those means of one of the parental breeds. The difference in the mean yields of both the protein and casein between the first generation and the Ayrshire breed (being the parental breed giving the higher yields of protein and casein) is not significant; while the difference between the two mean protein and casein yields of these four cows and the Shorthorn (being the parental breed giving the lower amount of protein and casein) is significant. It can be concluded that the mean protein and casein yields of the first generation may be expected to be nearer to the mean protein and casein yields of the parental breed giving the higher yields. This is in agreement with Ellinger (1923a) who worked with Red Danish and Jersey, their crosses and back crosses.

In the crosses he found that the fat yields of the individual approach more closely the average yield of the parental breed whose blood predominated. The F_1 generation gave yields intermediate between the two parental breeds but slightly nearer to the Red Danish. The progeny of each back cross was again mated to the same breed, with the result that the yields of these double back crosses to the Red Danish gave yields slightly higher than the Jerseys.

Variation in the protein and casein lactation yields of daughters of different bulls.

For milk yield the value of the bull may be estimated by the yields of his daughters. Comparison of two bulls is usually accomplished either by comparing the average yield of the daughters of each or by the degree of improvement of the yields of the daughters of each bull over the yields of the dams of those daughters or by a combination of these figures. Smith & Robison (1937) have shown that the magnitude of the variation in the yield of daughters should also be considered.

In the present investigation three bulls have a number of daughters varying from seven to twenty-one; four others having a lesser number of daughters (i.e. 4 - 5). The variation in the protein and casein yields of the daughters of these bulls was studied; but no final conclusions can be derived concerning the last four bulls since they have less than six daughters. Table XV shows the number of daughters, average yield and range in lbs. and the mean square difference between the yields of pairs of daughters of the same bull (derived by the method of pairing used by Smith & Robison, 1931).

TABLE XV.

Showing the variation in the protein yield of daughters of the same bull.

	Name of bull	No. of Daughters	Average protein yield in lb.	range (in lb.)	$\frac{\sum D^2}{N}$
(1)	Moorfield Call Boy	21	261	107-399	15222
(2)	Lyonston Douglas	7	320	249-389	4830
(3)	Barr Last Lap	7	295	195-354	6438
(4)	A.Royal Knight	5	341	295-384	2112
(5)	Royal Airman	5	205	109-245	6138
(6)	South Craig true line	5	324	230-382	6894
(7)	Chepelhill Emperor	4	422	398-470	2223

$\frac{\sum D^2}{N}$ = mean squared differences between the yields of protein of cows by the same sire.

All sires are Ayrshires except No. 5 which is a Beef Shorthorn.

From Table XV we conclude:-

(1) That the effect of each sire on the protein yields of his daughters is not equal to the effect of other sires on the protein yields of their daughters. This can be observed from comparing the figures of the last column.

(2) In case of the daughters of bulls Nos. 2, 4 and 7 - who are not only by the same sire but their mothers possess a common ancestor - this mean square difference is lower than in the case of cows whose only relationship comes through the sire.

(3) Arranging these sires according to their effect on the improvement of the protein yields of their daughters (excluding sires Nos. 2, 4, 7 because their daughters have connection through their dams), we find that Barr Last Lap and South Craig True Line are, undoubtedly, the best not only because the mean protein yields of their daughters are high but also because the variation in these yields is small. The improvement of the Ayrshire breed from the point of view of the protein yield should be laid upon such sires. By knowing the average protein yield of the daughters of these bulls we have a rough estimate for

the probable yield of protein to be secreted by any daughter even before she begins her lactation. On the other hand, sires such as Moorfield Call Boy are the worst from the viewpoint of the yields of protein of his daughters because of the large variation in the protein yields of his daughters, i.e. the highest yielder gives four times as much protein as the lowest under the same circumstances. The improvement of the breed cannot be entrusted to such bull.

TABLE XVI.

TABLE XVI.

Name of bull	No. of Daughters	Average casein yield in lbs.	Range (in lb.)	$\frac{\Sigma D^2}{N}$
(1) Moorfield Call Boy	21	224	86-333	11340
(2) Longston Douglas	7	258	197-314	4160
(3) Barr Last Lap	7	243	159-289	4268
(4) A Royal Knight	5	274	239-316	1582
(5) Royal Airman	5	167	88-204	4186
(6) South Craig True Line	5	269	201-309	3785
(7) Chapelhill Emperor	4	328	295-356	1273

(4) These results are substantially in accord with those obtained in respect of protein yield and the remarks concerning Table XV apply to Table XVI.

Statistical Methods.

Choice of a statistical method for the interpretation of the data proved to be a little difficult. Among the methods which may be used is the indirect correlation method used by Gowen. Although this gives figures denoting the correlation between the cows and their grandparents, it is open to criticism since direct correlation to the male ancestor is impossible. The figure used for the male ancestor is found theoretically by means of using the yields of the mother and daughters. Another defect in this method is that figures showing the correlation with male ancestors cannot be directly compared with those showing correlation to the female ancestors, since the one is based on phenotype and the other on genotype.

The other method of finding the mean of squared differences between the two individuals of each pair within every relationship group was found to be suitable for the present study. The principle of this method as advised by Fisher and explained by Smith and Robison (1931) is that "The closer the blood relationship between two individuals the more alike they should be.. In case of dairy cows, for instance, the closer they are related to each other the less difference should there be between their milk yields". A defect of this method is that there is no way up till now for finding the probable error of the figures obtained. On what basis should the error be calculated - the number of pairs, the number of cows or the number of ancestors of cows in each group? For testing the significance of the mean squared differences the z test is suggested by the writer.

If we arrange a table showing the different possible relationships between the cows - excluding the inter-generation relationships and tracing pedigrees only to the grandparents we find 24 groups of relationships. Only a few of these relationships are commonly in practice and some are very uncommon. This is because in-breeding is rare and the improvement is laid on the sire.

In Buchanan Smith & Robison's work about the inheritance of milk yield in the Ayrshire cows (1931), although they used great numbers, only six out of the 24 relationship groups yielded significant results.

Although some of the cows were analysed in another year no correction was done. Von Patow's (1925) "Byre Average" was not used. Although such correction seems to be necessary in some cases, it carries with it the serious disadvantage of the yields being "very much corrected" theoretically and thus we arrive at certain figures which might prove to be not suitable for the genetic study. Loertscher (1937) found that Von Patow's "Byre Average" is misleading. When applying this correction to his figures he arrived at the conclusion that 20 years' selection for high milk yield had apparently been ineffective. Such a conclusion does not agree with experience. Before using it the yields must be corrected for genetic variation, i.e. its use defeats its object.

The figure for the mean square difference between the paternal half sisters being high (Table XVII) may be due to the fact that one sire has twenty-one daughters. The same case was met before by Buchanan Smith (1937) while investigating the inheritance of milk yield in the Dairy Shorthorn cattle. He found that the mean square difference between the yields of cousins (i.e. cows by sires which are full brothers with dams unrelated), to be

TABLE XVII A.

Showing mean squared differences (between protein yields) and number of pairs for each relationship group.

Relationship of sires

Relationship of Dams.	Same		Unrelated	
	N (1)	$\frac{\Sigma D^2}{N}$ (2)	N	$\frac{\Sigma D^2}{N}$
Same	2	4658	8	8031
Paternal half sister	28	6448	51	9707
Unrelated	266	13165	3079	16720

Pairs of mother and daughter $\frac{\Sigma D^2}{N}$ = 8294 (N = 15)

(1) N = total number of pairs in each classification group.

(2) $\frac{\Sigma D^2}{N}$ = mean of summation of squared differences between the yields of pairs of cows in each group.

TABLE XVII B.

Showing table XVII A after adjusting the figure for the paternal half sister group.

Relationship of sires.

Relationship of Dams.	Same: N	Same: $\frac{\Sigma D^2}{N}$	Unrelated: N	Unrelated: $\frac{\Sigma D^2}{N}$
Same	2	4658	8	8031
Paternal half sister.	28	6448	51	9707
Unrelated	90 (69 + 21)	7975	3079	16720

Pairs of mothers and daughters 8294 (N = 15) even greater than that of unrelated groups. He gave the reason as due, perhaps, to the small number on which the figure is based and to the fact that a large number of pairs in that group traced to the same full brothers. This clearly shows that the failure to find a suitable method of calculating the probable error in this method is rather a serious defect.

It is possible that Buchanan Smith's figure would have been more in harmony with the trend of the rest of the figures, if the majority of the pairs were not traced to the same two - full brothers - sires.

In the present study the same difficulty was met. The figure for paternal half sisters (i.e. cows by the same sire and their dams are unrelated) was found to be high; though less than that of the unrelated group. It occurred to the writer to see what would happen to this figure if the most serious defect in it were eliminated, i.e. the majority of the pairs being traced to the same sire. The number of pairs by different sires was 69. To prevent the effect of any sire overwhelming the effect of the rest, a number of pairs should be added to this 69 pairs, taking that point into consideration. It is suggested here that the number of pairs tracing to the same ancestor should not exceed $\frac{1}{4}$ - $\frac{1}{3}$ of the total number of the group.

There remains one more point - which pairs of the biggest group should be added to that of the 69 pairs. One way is to take 25-30 pairs at random from the biggest group, and the other is to take the mean of the biggest group, multiply it with 25-30, add the figure obtained to the summation of the square differences of the 69 pairs, and divide the total by 90-95, taking the result as the index figure for the group.

When applying this method for the figure of the paternal half sisters - adding 21 pairs (this number was chosen because no other sire has more than 21 pairs in this group) to these 69 pairs - we find that the result is 7975 which is very near the index figure of the maternal half sisters group: (8031).

From Table XVII the following should be noted:-

(1) The figure for the unrelated group is much higher than any other figure in the table, i.e. being nearly double and in two cases more than double any of the rest of the figures.

(2) The figures for the maternal half sisters, and the paternal half sisters are very near each other.

(3) The figure for the group of the cows possessing the same sire and whose mothers are paternal half sisters is smaller than that of the paternal or maternal half sisters and the smallest in the table (excluding the figure for the full sisters).

(4) The figure for group possessing only one grand-parent in common (i.e. cows whose mothers are paternal half sisters and their sires are unrelated) is higher than the other four possessing one parent or more in common, but much lower than that for the unrelated group.

(5) It can therefore be concluded that, without exception, the differences grow bigger as the degree of kinship widen. This holds good also as regards casein (Tables XVIII A & B).

TABLE XVIII A.

TABLE XVIII.A

Showing mean squared differences (between casein yields) and number of pairs for each relationship group.

Relationship of Sires.

Relationship of Dams.	Same: N	Same: $\frac{\Sigma D^2}{N}$	Unrelated: N	Unrelated: $\frac{\Sigma D^2}{N}$
Same	2	2791	8	6718
Paternal half sister.	28	4465	51	7096
Unrelated	266	9695	3080	9944

Mother and daughter 6620 (15 pairs).

TABLE XVIII.B

Showing Table XVIII A. after adjusting the figure for the paternal half sister group.

Relationship of Sires.

Relationship of Dams.	Same: N	Same: $\frac{\Sigma D^2}{N}$	Unrelated: N	Unrelated: $\frac{\Sigma D^2}{N.}$
Same	2	2791	8	6718
Paternal half sister.	28	4465	51	7096
Unrelated	90 (69+21)	5769	3080	9944

The relative importance of the sire and dam.

Some early investigators, Fleishmann(1891), Hitcher (1899) Speir (1910) were of opinion that the sire has a greater influence on the quality of the offspring than the dam. Frolich (1930) agrees with them and points out the importance of the fat yield of the paternal grand-dam.

On the other hand a fewer number of investigators believe that the dam is more important. Hills and Boland (1913) and Rohwedder (1927) state that the female is prepotent in fat transmission. Gaines (1922) believes that performance can be transmitted through the female rather than the male.

However, the great majority of the recent investigators are agreed that the offspring is influenced equally by the sire and dam. Among the supporters of this opinion may be mentioned Gowen (1920, 1925, 1927), Ellinger (1923), Graves (1925), Copeland (1927) and Feige (1929).

All the last investigations were concerning the yields of milk or that of the butterfat. The result of the present investigation is that the sire and dam are of equal importance from the point of view of the transmitting ability of the genes governing protein and casein contents. This can be detected from the two figures showing the mean of the squared differences between the pairs in the group of paternal half sisters and that of the maternal half sisters.

If it is attempted to find out the coefficient of correlation for each group using the formula:-

$$r = 1 - \frac{\text{figure for group considered}}{\text{figure for unrelated group}}$$

the following would be found:-

Cows	(1)	full sisters	+.721
	(2)	having the same sire and their dams are paternal half sisters.	+.615
	(3)	having the same dam	+.52
	(4)	having the same sire	+.523
	(5)	whose mothers are paternal half sisters	+.419
	(6)	mother and daughter	+.505

The coefficients of correlation as found by this method denote only that the yields of the cows of a certain group are nearer to each other than the yields of cows of another group.

The inheritance of the percentage of protein was not carried out here since doing so would involve the consideration of two variables at the same time (i.e. milk yield and protein yield).

Buchanan Smith (1939) quoting Fairfield Smith (1936) states that the ratio of two characters can never supply more information than can be given by a consideration of both characters separately and that, as a rule, the information of a ratio is very much less than when each character is considered separately. He deduced that the ratio should never be used as the basis for methods of the improvement of plants. This deduction can equally well be applied to animals, and justifies the present tendency to desist from tracing the inheritance of percentage butterfat in dairy cattle. Buchanan Smith concludes that it is obviously sounder to concentrate upon the two components - total yield of milk and total yield of fat.

SUMMARY.

Details are given of the protein and casein yields during the complete lactation of healthy cows maintained under conditions of reasonably uniform management at the experimental farms of the Institute of Animal Genetics.

Methods of chemical analysis are stated in detail, with especial reference to the precipitation of the casein at its isoelectric point. The standard errors involved in the chemical work were determined experimentally and two methods for precipitating the casein were compared without finding a substantial difference.

An examination is made of the effect of various environmental conditions on the secretion of protein. Age of the cow was found to have a definite effect on the amount of protein secreted; it was found to increase with age. Service period also affects the amount, the longer the S.P. the bigger the amount of protein. Cows calving in October and November give the highest yields and those calving in March and April give the lowest. A dry period preceding the lactation has no effect if it exceeds three weeks.

Statistical methods used at present to investigate the problems of inheritance of the milk yield and its constituents are discussed in detail. The

squared difference method is employed and certain difficulties in its application are noted, requiring an adjustment of one of the raw figures to compensate for the fact that one relationship group is unduly dominated by the progeny of a single bull.

The results show that the amounts of protein and casein secreted during lactation are inherited. The effect of the sire was found to be equal to that of the dam in the transmission of the genes governing the inheritance of these two characters. The mean square difference between the yields of unrelated cows was greater than the mean square difference between the yields of any other relationship group. The mean square difference between the yields of dam and daughter was also found. Arising from this study certain correlations between the protein yield of related cows are included.

A P P E N D I X

I.

Showing particulars of the cows under examination and stating date of calving, age in months at calving, dry period preceding calving, service period and number of lactation.

In the case of crosses between breeds, the sire is the first-named :
B = Beef and D = Dairy.

APPENDIX I.

No.	Date of Calving	Age in months.	Service Period.	D.P.	Lactation.	Breed.
1	13.XII.37	35	136	-	1st	Ayrshire
2	2. X. 37	78	95	70	4th	"
3	31. X. 37	78	154	50	4th	"
4	28. X. 37	32	102	-	1st	"
5	11. X. 37	25	85	-	1st	Jersey
6.	2.XI. 37	50	77	66	2nd	Ayrshire
7.	30.IX. 37	32	107	-	1st	"
8.	25. X. 37	38	95	159	2nd	"
9.	24.IX. 37	47	90	62	2nd	"
10.	21. X. 37	35	162	-	1st	"
11.	8. X. 37	48	66	67	2nd	"
12.	31. X. 37	32	68	-	1st	"
13.	5.XI. 37	49	68	69	2nd	"
14.	22. X. 37	46	97	55	2nd	"
15.	10.XI. 37	38	72	172	2nd	"
16.	14.IX. 37	44	150	76	2nd	"
17.	20. X.37	34	101	-	1st	"
18.	23.IX. 37	44	60	40	2nd	"
19.	27.IX. 37	34	183	-	1st	"
20.	8. X. 37	33	152	-	1st	"
21.	2.XI. 37	49	73	66	2nd	"
22.	22.XI. 37	60	334	51	3rd	"
23.	6.XI. 37	78	115	85	3rd	"
24.	2.XI. 37	72	115	105	3rd	Shorthorn
25.	26.XII.37	78	214	212	3rd	Jersey
26.	26.XI. 37	58	85	153	3rd	Ayrshire
27.	I. X. 37	49	81	69	2nd	"
28.	6. X. 37	48	130	39	2nd	"
29.	28.XI. 37	50	146	43	2nd	"
30.	24.IX. 37	52	62	62	3rd	Jersey
31.	6. X. 37	30	92	-	1st	Ayrshire

APPENDIX I. (Contd.).

No.	Date of Calving	Age in months.	Service Period.	D.P.	Lactation.	Breed.
32.	16. X. 37	50	125	63	3rd	Ayrshire
33.	19. X. 37	43	174	–	1st	"
34.	28. X. 37	73	337	44	3rd	"
35.	15.IX. 37	40	203	39	2nd	"
36.	11. X. 37	44	201	67	2nd	"
37.	29.XII.37	48	161	88	2nd	"
38.	27.XII.37	63	133	86	3rd	"
39.	24.XI. 37	50	78	88	2nd	"
40.	12. I. 38	46	126	–	1st	Ayr/Guernsey
41.	13. I. 38	41	184	–	1st	Ayrshire
42.	14. I. 38	74	79	83	4th	"
43.	31. I. 38	57	146	135	2nd	Jersey
44.	1.11. 38	50	138	58	2nd	Ayrshire
45.	2.11. 38	37	Died	–	1st	"
46.	13.11. 38	75	74	113	4th	"
47.	14.11. 38	30	138	–	1st	"
48.	19.11. 38	88	110	42	5th	"
49.	18.11. 38	86	111	118	5th	"
50.	28.11. 38	88	105	128	5th	"
51.	28.11. 38	47	106	107	2nd	Ayr/B.Shorthorn
52.	1.111.38	62	92	171	3rd	Ayrshire
53.	3.111.38	47	76	202	2nd	"
54.	4.111.38	63	92	111	3rd	"
55.	17.111.38	84	93	201	4th	"
56.	8.111.38	46	82	230	2nd	B.Shorthorn/Ayr
57.	16.111.38	32	98	–	1st	Ayrshire
58.	19.111.38	69	81	225	4th	B/D Shorthorn
59.	20.111.38	30	101	–	1st	Ayrshire
60.	30.111.38	60	153	193	3rd	Jersey
61.	12. IV.38	97	232	94	6th	Ayrshire

APPENDIX I. (Contd.).

No.	Date of Calving	Age in months.	Service Period.	D.P.	Lactation.	Breed.
62.	13.IV.38	36	61	–	1st	Shorthorn/Ayrshire
63.	23.III.38	34	147	–	1st	Ayrshire/B.Short.
64.	4.IV. 38	46	58	212	2nd	Beef Shorthorn
65.	28.III.38	108	114	14	5th	Ayrshire
66.	12.IV. 38	41	60	–	1st	"
67.	16.IV. 38	70	112	185	4th	"
68.	14.IV. 38	97	128	75	5th	"
69.	29.III.38	59	86	221	3rd	"
70.	11.IV. 38	60	170	170	3rd	"
71.	27.IV. 38	100	144	109	6th	"
72.	30.IV. 38	73	64	112	4th	"
73.	27.IV. 38	38	99	–	1st	D.B.Short./Ayr.
74.	29.IV. 38	49	84	264	2nd	Ayrshire
75.	2. V. 38	31	96	–	1st	"
76.	29.IV. 38	32	119	–	1st	"
77.	29.IV.38	31	59	–	1st	"
78.	11. V. 38	70	92	295	4th	B.D.Shorthorn
79.	11.V. 38	65	111	102	3rd	Ayrshire
80.	14. V. 38	87	108	83	5th	"
81.	21. V. 38	87	141	90	5th	"
82.	26. V. 38	99	75	117	6th	"
83.	16. V. 38	78	–	254	4th	"
84.	19. V. 38	32	92	–	1st	D.B.Shorthorn
85.	30. V. 38	45	107	145	2nd	Ayrshire
86.	10.VI. 38	76	176	132	4th	"
87.	4.VII.38	40	73	–	1st	"
88.	15.VI. 38	62	43	–	1st	B.D.Shorthorn
89.	13.VI. 38	93	164	71	5th	Ayrshire
90.	17.VII.38	32	130	–	1st	"
91.	7.IX. 38	49	150	–	1st	B.D.Shorthorn
92.	9.XI. 38	33	98	–	1st	Ayrshire
93.	3. X. 38	32	106	–	1st	"

A P P E N D I X.

II.

Showing the percentage of protein in the milk of cows of different breeds for their complete lactations.

<u>Note</u>: These figures have been calculated by dividing the amount of protein secreted during the lactation by the total milk yield.

BREEDS.	% PROTEIN.
AYRSHIRES.	
C.Yellow Kate	3.19
Pries.Bina	3.16
Pries.W.Perfection	3.15
Pries.Bernice	2.79
Pries.Album	3.05
Pries.W.Stocks	2.84
Muncraig Ethel	2.89
Cockburn Bogie	3.26
Cockburn Constance	3.30
Shothead Greta	3.17
Shothead L.Lap	3.20
C.Mistress	3.16
Cockburn Dapple	2.96
Balgred.Jess	2.78
Balgred.O.Blossom	3.02
Balgred.Magpie	3.01
Balgred.Nessie	2.77
Cockburn Jess	2.87
Cockburn Countess	3.07
Cockburn Gladys	3.40
Cockburn Nessie	3.20
L.May Queen 4th	3.02
L.May Queen 3rd	2.91
Cockburn Roberta	3.09
Cockburn Cora	3.19
Cockburn Hilda	3.06
Cockburn Dosie	3.29
Cockburn Jeanette	3.20
Balgred.Countess	3.14
Auch.Bloomer	2.75
Auch.Buntie	2.92
Auch.Dandy	3.03
Auch.Ada	3.12
S.Caducia	2.92
A.Yellow Kate	2.72
A.Miss Craig	2.79
W.Vanity	3.34
C.Yvette	3.37
Cockburn Ada	2.93
Sh.Alannah	3.20
Sh.Blossom	3.05
Sh.Ethyl	3.06
Sh.Gretchen	3.03
Cockburn Daffodil	2.85
Cockburn Fanny	3.46
Cockburn Madge	3.02
Cockburn Missie	2.77
Cockburn Charlotte	3.04
Sh.Campanula	2.96
Sh.Patricia	3.60
M.Aster	3.16
S.Adeline	3.14
Shothead Pink	4.02
Shothead Hariett	3.07
Shothead Calyx	3.08
Shothead Henny	3.07
Shothead Shirley	3.16
Shothead Blonde	3.26
Doura Mona	3.10
Cockburn Nequita	2.94
Cockburn Fidget	3.29
Cockburn Florence	3.24
Lyonst.Dandy	2.96
Lyonst. Crummie	3.28
Lyonst. Brown Polly	2.95
Shothead Camilla	2.85
Caigton Pink	2.97
Lyonst. F.Beauty	3.09
Caigton Dewdrop	3.02
JERSEY.	
Light of F.Fairy	3.38
C.Calmette	3.55
Caldaria	3.32
L.N.Golden Lura	3.29
Manique	3.31
SHORTHORN.	
S.Rare Clipper	3.46
Meg Ramsay	3.05
S.P.Beauty	2.98
S.Telluria	3.32
S.Dark Lustre	3.36
CROSSES.	
Laps.Keepsake	3.21
Hecuba	3.00
Patsy	3.16
Claret's Cowslip	3.03
Claret's Sharon	3.02

MEANS.

Ayrshire	3.08
Jersey	3.36
Shorthorn	3.23
Cross	3.11

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PART II.

THE EFFECT OF SOME DISEASES AND ABNORMAL CONDITIONS ON THE QUANTITY AND QUALITY OF MILK.

INTRODUCTION.

The secretion of milk is a complicated process which may readily be thrown out of balance by any unfavourable factor, such as a decline in the general health of the animal, and more particularly, by any disease affecting the organs of secretion. This may result in a decline in the total yield with little or no change in the various constituents, or, what is more usual, a change in the proportions of these latter as well as in the total volume of the milk.

The opportunity was taken during the course of the present investigation to study the effect of mastitis on the quantity and quality of milk. Milk yield, butterfat, protein, casein, albumin and globulin percentages were available for these cows. Included in the study are cows of which one had post parturient dyspepsia and five were infected with mastitis, while the milk yield of the last two cows decreased owing to non-pathological causes.

I. MASTITIS.

Mastitis or mammitis is a term applied to the inflammation of the mammary glands and all types of infection of the udder irrespective of severity and cause are included under this head. When well marked changes in the milk result, the term "garget" is applied (Wooldridge, 1934).

Minett (1930) states that although severe cases of mastitis occur at times among first calf heifers the disease was distinctly more prevalent among cows over 5 or 6 years of age, which suggests that the disease tended to cause the greatest disturbance in cows which were reaching the peak of their milk producing capacity.

In studying the reduction of milk yield, Minett & Martin (1936) found that an average reduction of 954 lbs. of milk per lactation resulted from mastitis. In terms of percentages the loss was 10.8 among the Ayrshires, and among the Friesians of two herds 16.5 and 19.5 respectively. The effect of the disease upon the quality of the milk produced was of interest. Although milk from infected udders may be practically normal in appearance to the naked eye, it frequently had a somewhat salty and unpleasant taste. Moreover, because of this alteration in flavour, and owing to the fact that such milk sometimes does not undergo the normal lactic fermentation, the disease has acquired importance from the point of view of the cheese making and butter making industries.

Shaw & Beam (1935) studied the effect of mastitis upon milk production. Four quarters of the udder were examined separately, so that any differences between the healthy quarters, and also the differences between healthy quarters and infected quarters could be observed. They concluded that only a small variation exists between the pounds of milk, per cent butterfat and pounds of butterfat produced by opposite non-infected quarters of a cow's udder, but that a considerable variation may be found between the production of opposite quarters of the udder where one of these is infected with mastitis, while the other is normal. They found also that mastitis infection reduced the milk production approximately 22 per cent and the butterfat production 24 per cent, after allowing for the maximum variation found in the milk and butterfat production of non-infected quarters.

Cranfield & Ling (1929) analysed milk from a cow which secreted abnormal milk for three lactations and found that the ratio of calcium to phosphorus varied from .78 to 1.65.

Koestler (1920) (reported by Shaw & Beam 1935) found that the abnormalities in secretion were accompanied by changes in composition which followed a general rule. The amounts of globulin, chlorine, sodium and sulphate increased while the lactose, potassium, magnesium, calcium and phosphorus content decreased.

It was shown (Minett 1930) that mastitis can be caused by different kinds of organisms under the groups streptococci, staphylococci, C. pyogenes and gram-negative bacteria belonging to E. coli or related groups.

An indirect method for the diagnosis of mastitis has been discovered, based upon the change in the casein numbers of the milk drawn from the quarters of the udder which respectively are, and are not, affected by the disease.

Rowland (1938) found that the percentage of $\frac{C.N.}{T.N.}$ in mastitis samples is lower than that of the normal milk, for samples drawn from individual quarters. Rowland's method can be properly applied only on samples from individual quarters. All the indirect methods have the advantage of simplicity, although they give no sure indication of infection.

In this research cases of mastitis were detected in the laboratory by chemical methods alone, long before they were observed on the farm. In one case a year elapsed after the detection of disease in the cow by chemical methods before it was confirmed by bacteriological diagnosis.

In the present investigation, records of the whole lactation showing milk yield, percentages of different proteins, percentages of butter-fat and solids-not-fat were available for 5 cows infected with mastitis. It was thought desirable to analyse each case alone before drawing the general conclusions. It should be mentioned that the method used for extraction of the casein number is approximate, and that the casein number (for mastitis samples) thus found is always higher than that obtained by estimating the total nitrogen and the non protein nitrogen (usually 6% of the total nitrogen in normal milk, but invariably higher in samples obtained from cows suffering from mastitis), and then calculating the casein number.

(a) Linnhead Hilda 3rd: An Ayrshire cow which calved on 13th February, 1938. At the start of her fourth lactation she was in normal health and chemical analysis of her milk on 24th February and 10th March showed nothing unusual; the casein number for each analysis was 76.8. At the third analysis done on 24th March it was found that the percentages of both protein and casein had fallen sharply. The casein (when found approximately from the formula $\frac{\text{casein nitrogen}}{\text{total protein nitrogen}} \times 100 \times \frac{94}{100}$) was found to be 74.5, i.e. a little below the minimum for healthy cows. At the 7th April the percentages were still low and the casein number was less (70.7). Enquiries from the farm revealed that no abnormalities had been noticed on veterinary inspection. The cow continued to give milk with low casein number, which rose after some time to the normal standard of uninfected cows. Early in May the usual symptoms of streptococcic mastitis were observed, the fore milk showing distinct clotting, but with no decrease in total yield of milk. On subsequent chemical analysis the casein number increased.

On the 8th July the casein number was determined in two different ways (1) theoretically by the formula $\frac{\text{Casein N.}}{\text{T.P.N.}} \times \frac{100 \times 94}{100}$; (2) experimentally, by duplicate tests of the casein nitrogen and the total nitrogen. The theoretical figure for the casein number was 75.8, the actual figure was 70.7. Hence, in this particular case, the N.P.N. instead of being 6%, as normally, was considerably higher.

A bacteriological examination of the milk drawn on 25th July stated that "Infection in this case appears to be due to group I streptococcus", i.e.

streptococcus agalactiae, the usual cause of sub-clinical mastitis. Table I shows chemical analysis of milk of this cow.

TABLE I.

EFFECT OF MASTITIS.

TABLE I.

Record of cow: Linnhead Hilda 3rd.

Date	Milk yield.	T.P.N.	C.N.	A. + G.N.	C.no (approximate)	% F.
		mg. per 100 gm. milk.				
24.II.38	52	456	373	83	76.8	3.23
10.III.38	50	488	399	89	76.8	3.48
24.III.38.	52.5	357	283	75	74.5*	3.18
7. IV.38	46.25	371	279	92	70.7*	3.87
21. IV.38	44.5	381	291	90	71.8	3.30
9. V.38	43.5	444	343	101	72.6	3.36
30. V.38.	41.5	442	345	97	73.4	3.11
20. VI.38	39	403	331	71	77.2	2.56
8.VII.38.	37.5	416	332	84	75.1	4.05
25.VII.38	31	389	311	78	75.2	4.20
1.VIII.38	35.5	397	288	109	68.2	3.46
16.VIII.38	30.5	415	317	98	71.8	—
6. IX.38.	30.25	436	333	103	71.8	3.43
27. IX.38.	16.5	511	392	118	72.1	4.06
18. X. 38	7.5	609	444	165	68.0	3.47

* Observe the sharp decrease in Protein, casein and casein no.

(b) Cockburn Marjorie: An Ayrshire cow, which calved for her second lactation on 23rd September, 1937. During the following December the casein number was very low (70-72). On 20th December a bacteriological examination was carried out and "no evidence of infection" was found. The cow continued to give milk with low casein number.

During her next lactation, i.e. during December 1938 the result of a bacteriological examination was positive. No chemical examination was carried out on her milk during the third lactation. See Table II.

TABLE II.

EFFECT OF MASTITIS.

TABLE II.

Record of cow: Cockburn Marjorie.

Date	Milk yield.	T.P.N.	C.N.	A. + G.N.	Casein No.	% Fat
		mg. per 100 gm. milk.				
6. X.37	42	518	429	89	77.9	-
13. X.37	45.5	464	363	101	73.5	-
20. X.37	40	412	342	70	78.0	3.8
27. X.37	37.5	438	337	101	72.3	3.49
3. XI.37	35.5	449	-	-	-	3.55
17. XI. 37	33	485	377	108	73.1	3.33
1.XII.37	29.5	488	377	111	72.6	3.29
14.XII.37	30	494	371	123	70.6	3.96
28.XII.37	32	501	373	128	70.0	3.47
11. I.38	28.5	508	406	102	75.1	3.16
25. I.38	25	475	383	92	75.8	3.20
8.II.38	26.5	457	354	103	72.8	3.47
22.II.38	23	471	364	107	72.7	3.30
8.III.38	21.5	475	358	116	70.9	3.40
22.III.38	22	439	346	93	74.1	2.86
12.IV.38	19.5	447	358	90	75.3	3.44
3.V. 38	17.5	480	365	115	71.5	3.43
24. V.38	12.5	463	351	112	71.2	3.76
14. V.38	7	510	371	140	68.4	3.14

(c) S. Proud Lustre: A Beef/Dairy Shorthorn cow which calved on 19th March for her fourth lactation. She had a very short lactation. The percentage of both the protein and casein nitrogen fell with the advancement of the lactation. The casein number was always low and steadily decreased. Full particulars may be found in Table III.

Two bacteriological tests were carried out on the milk of this cow. The first was on 2nd May 1938 when "no evidence of infection was found". The second test was made on 27th June, 1938, i.e. nearly two months later; group I Streptococci were then found.

The negative result found in the first test, as also in the case of Cockburn Marjorie, was probably due to the intermittent excretion of the infective organism.

The casein number denotes that this cow was infected from the beginning of her lactation (Table III.).

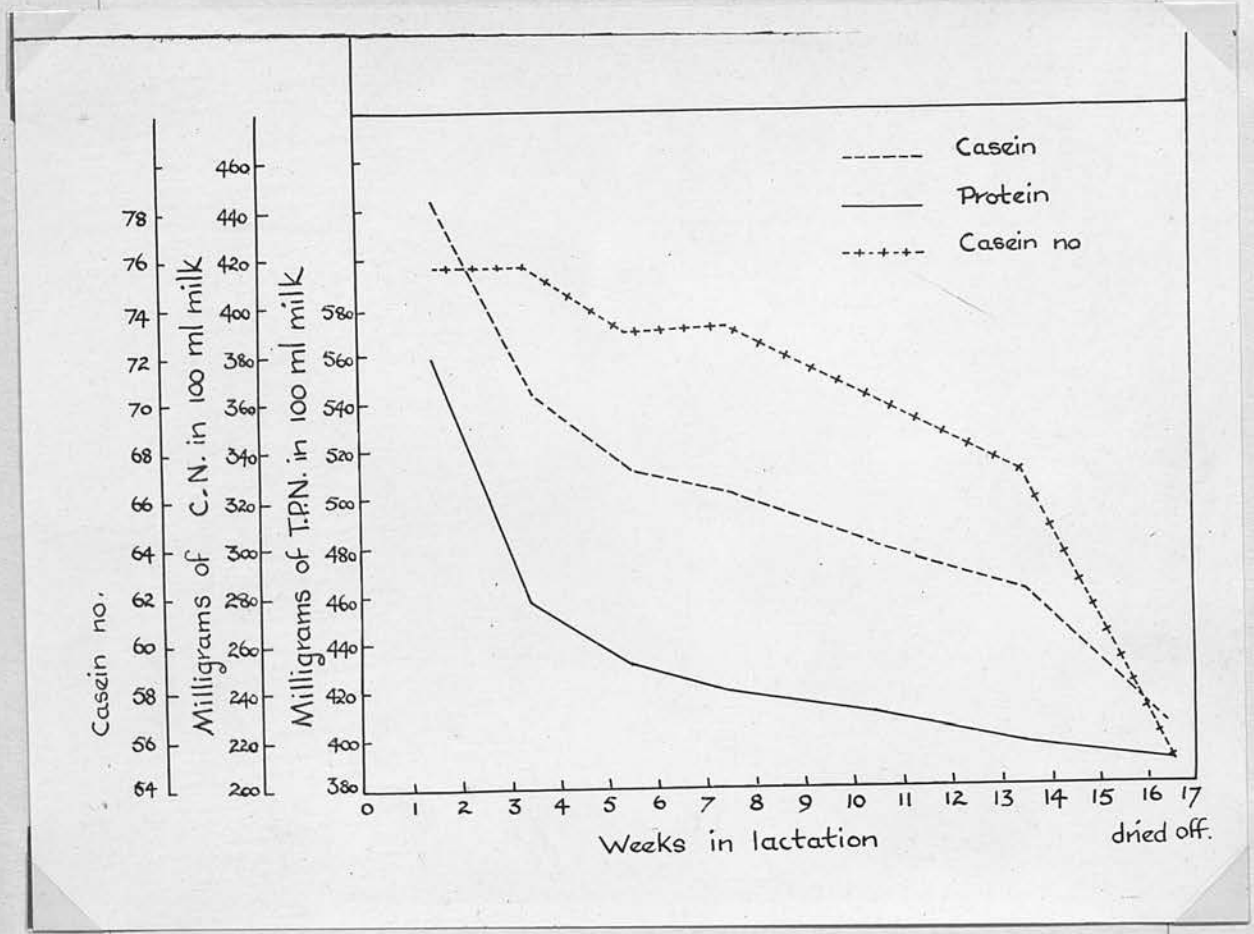
TABLE III.

EFFECT OF MASTITIS.

TABLE III.

Record of cow: S. Proud Lustre.

Date	Milk yield.	T.P.N.	C.N.	A. + G.N.	Casein No.	% Fat
		mg. per 100 gm. milk				
28.III.38	24.5	559	444	115	74.7	3.39
15.IV.38	29.5	457	364	93	74.9	3.15
29.IV. 38	29.5	432	332	100	72.3	4.40
11. V. 38	21	420	323	97	72.3	3.38
1. VI.38	14.5	410	302	108	69.2	2.34
22. VI.38	11.5	398	281	117	66.4	3.13
13.VII.38	3.5	389	221	168	53.4	5.43



GRAPH I.

Showing the curve for protein, casein percentages and casein number of cow S.Proud Lustre during her fourth lactation.

(d) Eaton Rosebud: A Dairy Shorthorn cow in her third lactation. Although the samples were taken from the four-quarters mixed milk, the casein number was lower than that of healthy cows. The bacteriological test confirmed the result of the chemical analysis.

TABLE IV.

EFFECT OF MASTITIS.

TABLE IV.

Record of Cow: Eaton Rosebud.

Date	Milk yield.	T.P.N.	C.N.	A. + G.N.	Casein No.	% Fat
		mg. per 100 gm. milk				
10.XI. 37	29.5	607	457	150	70.8	4.71
23.XI. 37	31.5	603	470	133	73.3	4.79
8.XII.37	36.5	534	422	112	74.3	3.23
22.XII.37	29.5	503	387	116	72.3	3.46
5. I. 38	29	512	409	103	75.1	3.04
19. I. 38	28.5	508	400	108	74.	3.23
2.II. 38	26.25	473	370	103	73.5	3.32
16.II. 38	21	484	376	108	73	3.33
2.III.38	14	492	405	88	77.4	3.71
18.III.38	15.5	516	428	88	78	2.97
6.IV. 38	14	487	397	90	75.7	3.43
27.IV. 38	8.5	502	404	98	75.7	3.65
18. V. 38	13.5	478	387	91	76.1	3.18
8.VI. 38	8	477	373	105	73.5	3.62

(e) Cockburn Celandine: An Ayrshire cow in her second lactation. Although no bacteriological test was carried out, it was considered infected since her casein number is very low (Rowland, 1938).

TABLE V.

EFFECT OF MASTITIS.

TABLE V.

Record of cow: Cockburn Celandine.

Date	Milk yield	T.P.N.	C.N.	A + G.N.	Casein No.	% Fat
		Mg. per 100 grams of milk				
9.XI. 37	43.75	567	465	102	77.1	3.77
23.XI. 37	50.5	452	361	91	75.9	3.52
7.XII.37	45	443	351	91	74.5	3.44
21.XII.37	38.5	441	340	100	72.5	3.19
4. I. 38	34	440	336	104	71.8	3.50
18. I. 38	35.5	436	334	102	72	3.32
1.II. 38	33.5	441	337	103	71.9	3.46
15.II. 38	33	439	344	95	73.7	3.30
I.III. 38	32	466	353	113	71.2	3.06
15.III.38	30	456	340	116	70.	3.67
5.IV. 38	24.5	451	347	104	72.3	3.96
26.IV.38	25.5	462	356	106	72.5	3.72
17. V. 38	21.5	444	331	114	70	3.58
7.VI. 38	19.5	479	358	121	70.4	3.49
28.VI. 38	13	516	384	133	69.9	3.15
19.VII.38	8	511	372	139	68.4	3.12

DISCUSSION AND CONCLUSIONS.

It was shown (Minett & Martin, 1936, Shaw & Beam, 1935) that mastitis causes a decrease in the milk yield; and it was demonstrated by Rowland (1938) that it affects the casein number. The result of the present study confirms that of Rowland. In some cases, not only the casein number decreased but the percentage of protein and casein was also lowered.

We may conclude that for the problems of the inheritance of milk yield, mastitis infected cows must be excluded from a statistical analysis since there is another important factor governing the milk yield in this case (i.e. disease) other than the hereditary ability which is the subject of study and the environmental factors which could be taken into consideration before the comparison takes place.

The same considerations will apply to the question of protein and casein inheritance, for not only is the total quantity of protein diminished *pari passu* with the diminished yield of milk (Azarme, 1938; S. Zein-El-Dine, 1939) but the relative casein content of the milk in diseased conditions has been shown to decrease.

It has been shown (M. Zein-El-Dine, 1939; Minett, 1930) that the occurrence of this disease is very common among the British herds. If those who studied the inheritance of milk yield arrived at positive results, this should not be taken as an indication that the effect of the disease on the milk yield is of no importance. All that can be said is that perhaps only few cows with the disease at its early stages of development were included, and the great number of normal animals used in the statistical analysis overshadowed the effect of the few diseased ones.

In the present study it has been reported that one year elapsed between the mastitis detection by the chemical method and by bacteriological examination. This shows that the bacteriological method, although it gives better results and definitely identifies the infecting organism, is not efficient in every case and that the indirect method should be applied side by side with it as confirmation test. The cause of the first bacteriological test in the case of Shothead Proud Lustre being negative is supposed to be due to the "intermittent excretion of the organisms".

In the case of cow L. Hilda the casein number as found theoretically differed very much from the actual value found experimentally. In the first theoretical calculation the percentage of the non-protein nitrogen was assumed to be 6% only (as in the normal milk). Comparison with the experimental figure shows that the percentage of N.P.N. in this sample is unusually high. This confirms Davies (1933) who found that in samples derived from cows giving milk with low solids-not-fat (most probably mastitis cows) the protein reached a minimum of 85% of the total nitrogen, thus giving a percentage of N.P.N. in his case of 15%, i.e. 250% of normal. Many theories have been put forward to explain this phenomenon. The most acceptable one is that owing to the effect of the disease, the udder cells will not be able to synthesise casein with the same speed as healthy cells and cannot govern the filtration of the precursors of the nitrogen constituents of the milk. The result is milk low in casein and high in the other nitrogenous fractions (Espe, 1938).

The normal curve for protein percentage and also for casein percentage is high at the time of calving and then declines until about the fourth week, after which it rises gently for the rest of the lactation (Azarme, 1938). In the case of cow S. Proud Lustre the curve for protein begins moderately high and goes down steadily throughout the lactation. It has been demonstrated that the milk yield is negatively correlated with both the protein and casein percentages (Azarme, 1938). As the percentages of both casein and protein diminish while the volume of milk is also decreasing, it is questionable whether this cow can be regarded as normal.

In the event of Creameries purchasing milk for cheese manufacture on the basis of casein content, the dairy farmer, whose herd is affected with mastitis, may suffer considerable loss. Not only will he lose the difference in milk yield between the actual amount of milk that his cows give and the probable amount that would have been secreted, but also receives a lower price per unit, owing to the diminished percentage of casein in the milk.

Cheese and butter factories which buy milk on liquid measure from infected herds experience some loss due to the fact that this milk is low in casein, and does not undergo the usual lactic fermentation, which is quite important for buttermaking.

It should be noted that any quarter of the udder may be infected without influencing the others, because each quarter is a completely independent gland (Proks 1928, Benton 1929, and Mattick & Hallett 1929). So we might find some cows reacting to the bacteriological test and do not show a drop in casein number if the sample is taken from the four quarters' mixed milk.

II. ACETONURIA.

During the present investigation only one case of acetonuria was observed. In this case, the milk yield of an Ayrshire cow (C. Jess) dropped from 42.25 lb. to 28.5 lb. in seven days and increased again to 38 lb. after another week. This happened one month after parturition. The rest of the lactation was quite normal. The protein, casein, albumin and globulin percentages were high, the casein number was low (when the cow gave 28.5 lb. of milk). The different protein percentages dropped after that and the curve was resumed in the normal way. The casein number for this cow was normal (over 78). It must be mentioned that these determinations were not done in duplicate. This cow was a good milker and the lactation lasted for 12 months exactly. This case occurred during October, 1937. The service period was 150 days.

N.B. The service period was mentioned here because it has been shown by Sanders (1927) that the length of lactation depends, to a great extent, on the service period.

Table VI shows the record of this cow.

EFFECT OF ACETONURIA.

TABLE VI.

Record of cow: Cockburn Jess.

Date	Milk yield.	T.P.N.	C.N.	A. + G.N.	Casein No.	% Fat
		Mg. per 100 grams of milk				
5. X. 37	42.25	463	398	65	80.8	4.09
12.X. 37	28.5	480	375	104	73.4	–
19.X. 37	38	416	344	72	77.7	3.97
25.X. 37	35.5	403	333	70	77.6	3.30
I.XI.37	35.5	396	330	65	78.8	3.35
15.XI.37	45.5	415	336	79	76.1	3.80
2.XII.37	39	412	346	66	78.9	3.51
15.XII.37	47	429	355	74	77.8	2.51
29.XII.37	41	454	372	82	77.	3.75
12.I. 38	46	436	371	65	80.	3.35
26.I. 38	46.5	427	367	60	80.8	3.16
9.II.38	45.75	420	356	64	79.7	3.04
23.II.38	44	409	345	64	79.2	3.32
9.III.38	44.5	431	354	77	77.2	3.42
23.III.38	42.5	435	366	69	79.1	3.25
13.IV.38	40	438	368	70	79.	–
4. V.38	39.5	469	393	76	78.8	3.70
25. V.38	37.5	455	381	74	78.7	3.39
14.VI.38	36.5	488	413	75	79.6	3.51
4.VII.38	31	498	414	85	78.2	4.07
26.VII.38	22	537	419	119	73.3	3.73
11.VIII.38	17.5	511	397	113	73.	4.46
I.IX.38	9	665	494	171	69.9	4.89

III. THE EFFECTS OF SOME ABNORMAL CONDITIONS.

In some cases a sharp decline in the milk yield cannot be traced to any disease. It was thought that it would be of interest to investigate the effect of some causes other than diseases on the quantity and quality of the milk.

During the present study an Ayrshire cow received udder injuries through being gored. The milk yield dropped about 74% and the percentages of protein, casein and fat increased abnormally; although the milk yield declined from 40.5 lb. to 10.5 lb., the increase in the percentages of the different milk constituents was comparatively moderate. The effect was temporary and after 4-5 weeks the quantity and the composition of milk returned to normal.

Table VII shows the chemical analysis of this cow's milk through her lactation.

EFFECT OF ABNORMAL CONDITIONS.

TABLE VII.

Record of cow: Priestlands Album.

Date	Milk yield	T.P.N.	C. N.	A + G.N.	Casein No.	% F.
		Mg. per 100 grams milk				
6 V 38	55	449	370	79	77.5	3.75
20 V 38	52	447	356	91	74.9	-
3 VI 38	47.5	497	400	97	75.7	-
16 VI 38	51	494	396	98	75.4	-
27. VI 38	44	500	411	89	77.3	3.5
18.VII 38	44.5	488	402	86	77.4	3.6
8 VIII 38	40.5	498	398	100	75.2	3.8
29 VIII 38	10.5	584	451	133	72.6	7.9
19 IX 38	26.5	538	426	112	74.4	4.56
10 X 38	22	527	417	110	74.4	3.68
31 X 38	18	510	404	106	74.4	1.89
22 XI 38	11.5	550	429	121	73.3	2.61
6 XII 38	10	530	432	98	76.6	2.3
21 XII 38	10	542	426	116	73.9	2.7

There was a Shorthorn cow (Shothead Rare Clipper) that did not consume all her ration at a certain period during the lactation. The percentage decrease in the yield or the percentage increase in the proteins or butterfat cannot be accurately calculated because it is not known definitely when the reduction began since it took place rather slowly.

Table VIII shows the results of the chemical analysis of this cow's milk through her lactation. The milk yield increased after some time but did not return to the original level.

EFFECT OF ABNORMAL CONDITIONS.

TABLE VIII.

Record of Cow: S. Rare Clipper.

Date	Milk Yield	T.P.N.	C.N.	A. + C.N.	Casein No.	% Fat
		Mg. per 100 grams of milk				
27 V 38	10.5	530	428	102	75.9	5.62
10 VI 38	13.5	505	425	80	79.1	3.18
24 VI 38	17.5	503	405	98	75.7	3.49
7 VII 38	17	466	381	84	76.8	4.00
28.VII 38	16	468	405	64	81.3	3.81
15.VIII 38	15	524	437	87	78.4	2.60
5. IX 38	14	543	456	87	78.9	3.36
26 IX 38	10.5	559	469	90	78.8	3.71
17 X 38	11	548	443	105	76.0	3.73
7.XI 38	6.5	556	455	101	76.9	4.92
24 XI 38	7.5	535	469	66	82.4	4.00
15 XII 38	11.25	562	464	98	77.6	4.44
9 I 39	10.75	556	467	89	78.9	3.85
30 I 39	10.75	566	474	92	78.7	2.85
20 II 39	11.25	573	468	105	76.8	3.9

THE EFFECT OF OESTRUM ON MILK.

Snapp (1930) states that when the cow is on heat, the milk yield usually declines. McCandlish (1935) found that the effect of oestrum on the milk yield and butterfat percentage is indefinite; sometimes it results in an increase, and sometimes in a decrease of the fat percentage. Azarme (1938) observed an increase in the percentage of total protein nitrogen in the milk of cows in which the milk yield fell during sexual excitement.

During the present work, a case was observed in which the protein and casein percentages fell during the sexual excitement as well as milk yield.

Table IX shows the part of the lactation table concerning this.

TABLE IX.

Date	Milk yield.	% T.P.N.	% C.N.	% Fat.
24.3.38	19	510	432	5.05
8.4.38	11.5	467	405	3.83
21.4.38	16.5	512	425	3.82

This case is reported because of the unusual behaviour of the protein and casein percentages.

In order to determine the effect of oestrum in cows on the percentages of protein, analyses of the morning and evening milk were performed separately to determine the percentages of protein and casein. The first sample was taken from the well mixed milk that was given by the cow at the first milking after she began to show signs of heat. At each subsequent milking a sample was taken up to a total of six milkings covering a period of three days.

The results of the analyses are shown in Tables X and XI.

TABLE X.

Effect of heat (oestrum) on the milk and percentages of Protein and Casein.

Name of Cow	I a	I b	I c	II a	II b	II c	III a	III b	III c	IV a	IV b	IV c	V a	V b	V c	VI a	VI b	VI c
C. Irene	15	435	367	15	409	346	16	440	360	15.75	–	–	16	458	387	15.5	457	384
C. Bogie	21	449	369	22	360	354	23	453	371	–	–	–	21	460	393	20	478	401
C. Constance	21	473	389	23.5	442	365	23	444	377	22	449	370	23	468	398			
C. Adele	15.5	419	375	14.5	426	357	15.5	432	364	16	425	364	15	460	375	16	–	378
C. Dosie	20	466	403	20	465	386	17.5	497	413	18.5	496	396	16.5	506	424	16	500	423
W. Vanity	15.5	484	413	16.5	481	402	18	479	395	16	491	413	18.5	479	406			
C. Catherine	19.5	463	382	19.5	445	371	19	446	373	19.5	–	403						
C. Cadence	18	415	344	21.5	374	315	20	387	322	19	391	331						
Means	18.2	450	380	19.1	437	362	19	447	377	18.5	450	380	18.3	472	396	16.9	478	397

I, II, III..etc are the first, second, third... samples after occurence of heat

a .... milk yield in lb.

b .... T.P.N. in mg. in 100 gm. milk

c ..... C.N. in " " " "

TABLE XI.

Summary of the results of the analysis of milk obtained from cows in oestrum.

	Milk yield. (lb. per 24 hours)	Mgm. of protein nitrogen in 100 gm. of milk.	Mgm. of casein nitrogen in 100 gm. ~~in~~ of milk.	No. of samples analysed.	
				a.m.	p.m.
1st sample	18.2	450	380	3	5
2nd "	19.1	437	362	5	3
3rd "	19	447	377	3	5
4th "	18.5	450	380	4	2
5th "	18.3	472	396	3	3
6th "	16.9	478	397	3	1

Table XI shows that the oestrum had no significant effect on the milk yield. In some cases the milk yield dropped when the cow was milked after or during the sexual excitement but this was usually due to the cow "holding up" her milk, and the next milking was higher in such a case. In this table it is necessary to compare samples 1, 3 and 5 since the samples were arranged not owing to the time of milking (morning and evening) but to the time of the occurrence of the oestrum. Expectations that the milk would be lower in quantity and higher in percentage of proteins in the first samples than in the second samples were verified. The difference in the percentages of protein and casein in the first samples as compared with the second samples is small and can be attributed to the first samples containing more evening milk. From this work it may be concluded that the oestrum has little or no effect at all on the quantity and quality of milk yielded.

A NOTE ON THE CONSTANCY OF THE CASEIN NUMBER DURING LACTATION.

Rowland (1938) found that the determination of casein number can be used as an indirect method for diagnosis of mastitis. This would be true in all cases provided the casein number does not vary, or varies within very narrow limits, during the whole lactation. The casein number is usually constant during the whole lactation except for two periods, the beginning and the end. During the first week of lactation, particularly in the first three days, the casein number is lower than that given by Rowland. During the course of this study a peculiar anomaly was observed: some healthy cows secreting milk with high casein number during lactation and giving negative results to the bacteriological diagnosis for mastitis were found to secrete milk with low casein number near the end of their lactations. This does not mean that every cow secretes milk with low casein number near the end of the lactation, for there are many cows in which the casein number did not drop. This fall of the casein number in general may be attributed to one of the following causes:-

(1) The quantity of casein synthesised by the parenchymatous tissues remains constant but the udder secretes more A. + G. + N.P. nitrogen.

(2) The proportions of the different milk proteins remain the same but for some reason the udder cells become permeable to the blood serum (e.g. because of some disease).

(3) The quantity of the casein secreted may be high but owing to the disproportionately great quantities of Albumin and Globulin simultaneously secreted the casein number will be low (e.g. in case of newly calved cows).

(4) The percentage of casein becomes high but at the same time the percentage of A. + G.N. rises still more (e.g. in such cows nearing the end of their lactation).

It should not be assumed that every cow secretes milk with low casein number near the end of her lactation. As the table shows there are cows which secrete milk with normal casein number up to the last day of milking.

This shows that the casein number cannot be used as a criterion for the diagnosis of mastitis towards the end of lactation.

Weeks before end of Lactation

TABLE A.

Name of Cow	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
A.B.	-	-	-	74.6	-	-	75.1	-	-	75.8	-	75.5	-	-	-	76.0	-	-	69.7
S. Blos.	-	-	-	-	-	81.4	-	77.7	-	75.4	-	75.2	-	76.1	-	-	76.8	-	-
L.B.Poll	-	-	77.5	-	-	77.6	-	-	77.1	-	-	74.9	-	-	77.8	-	73.8	-	-
C. Daff.	-	-	-	80.2	-	80.5	-	-	-	79.5	-	77.5	-	-	77.5	-	-	74.7	-
S. Ethyl	-	76.7	-	-	76.4	-	-	76.5	-	-	77.3	-	-	77.9	-	-	72.7	-	70.0
C.Glad	-	78.6	-	-	75.5	-	-	73.3	-	-	76.3	-	-	70.9	-	73.4	-	-	66.4
S. Gret	-	-	-	-	79.7	-	76.5	-	74.9	-	76.2	-	-	73.6	-	-	68.9	-	-
C. Jess	78.8	-	-	78.7	-	-	79.6	-	-	78.2	-	-	73.3	-	73.0	-	-	69.8	-
C.Jeanet	-	-	-	-	75.0	-	74.5	-	-	74.0	-	-	74.2	-	-	72.7	-	-	72.8
L. Crum	-	-	-	-	75.5	-	-	75.5	-	-	74.6	-	-	73.4	-	-	73.7	-	67.6
L. ofF.F	-	-	-	75.1	-	-	74.7	-	-	73.2	-	-	74.8	-	73.6	-	-	-	68.5
C.Madge	-	78.1	-	-	78.1	-	-	78.9	-	77.9	-	-	-	77.5	-	77.8	-	-	76.7
C. Neq	-	-	78.9	-	-	78.2	-	-	77.3	-	-	76.8	-	74.7	-	-	75.8	-	-
C. Cons.	-	-	78.2	-	-	77.1	-	-	77.3	-	-	79.7	-	-	75.2	-	74.5	-	-
C. Dosie	-	-	79.3	-	78.9	-	-	79.3	-	-	80.0	-	-	76.3	-	-	73.3	-	-
W. Vani	-	-	77.6	-	-	79.3	-	-	79.6	-	75.6	-	-	-	78.0	-	77.3	-	-
Patsy	-	-	-	-	78.3	-	-	76.8	-	-	75.1	-	76.8	-	-	78.7	-	-	72.6
S. Adel	-	-	-	-	75.4	-	78.2	-	-	76.3	-	-	75.6	-	-	78.8	-	-	76.9
S. Greta	-	-	-	78.9	-	-	80.5	-	-	78.4	-	-	74.3	-	-	78.5	-	-	77.5
D. Mona	-	-	-	75.5	-	-	77.1	-	-	77.5	-	-	72.0	-	-	74.2	-	-	69.9

Showing the Casein number for the last 18 weeks before end of lactation.

Weeks before end of Lactation

TABLE A.

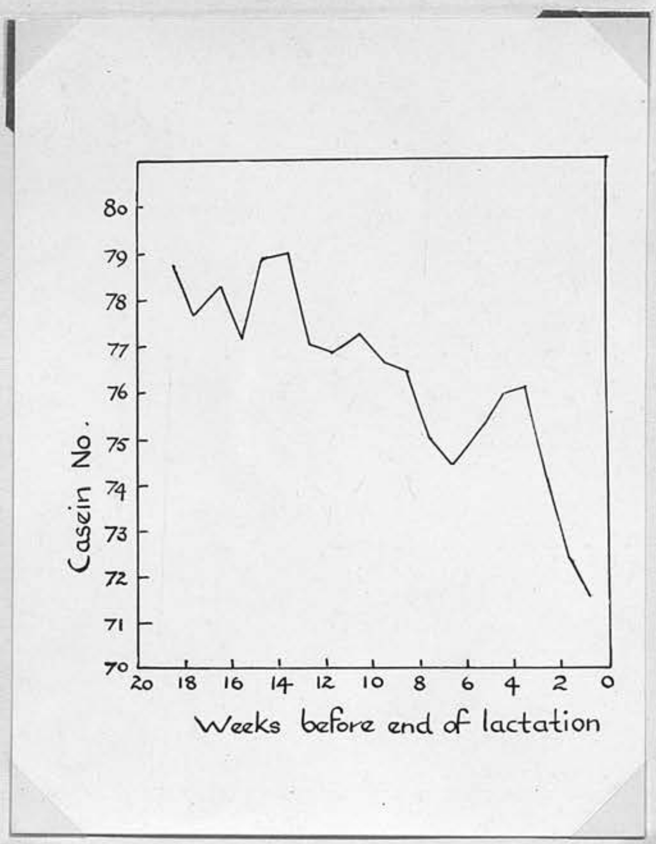
Name of Cow	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
A.B.	-	-	-	74.6	-	-	75.1	-	-	75.8	-	75.5	-	-	-	76.0	-	-	69.7
S. Blos.	-	-	-	-	-	81.4	-	77.7	-	75.4	-	75.2	-	76.1	-	-	76.8	-	-
L.B.Poll	-	-	77.5	-	-	77.6	-	-	77.1	-	-	74.9	-	-	77.8	-	73.8	-	-
C. Daff.	-	-	-	80.2	-	80.5	-	-	-	79.5	-	77.5	-	-	77.5	-	-	74.7	-
S. Ethyl	-	76.7	-	-	76.4	-	-	76.5	-	-	77.3	-	-	77.9	-	-	72.7	-	70.0
C.Glad	-	78.6	-	-	75.5	-	-	73.3	-	-	76.3	-	-	70.9	-	73.4	-	-	66.4
S. Gret	-	-	-	-	79.7	-	76.5	-	74.9	-	76.2	-	-	73.6	-	-	68.9	-	-
C. Jess	78.8	-	-	78.7	-	-	79.6	-	-	78.2	-	-	73.3	-	73.0	-	-	69.8	-
C.Jeanet	-	-	-	-	75.0	-	74.5	-	-	74.0	-	-	74.2	-	-	72.7	-	-	72.8
L. Crum	-	-	-	-	75.5	-	-	75.5	-	-	74.6	-	-	73.4	-	-	73.7	-	67.6
L. offF.F	-	-	-	75.1	-	-	74.7	-	-	73.2	-	-	74.8	-	73.6	-	-	-	68.5
C.Madge	-	78.1	-	-	78.1	-	-	78.9	-	77.9	-	-	-	77.5	-	77.8	-	-	76.7
C. Neq	-	-	78.9	-	-	78.2	-	-	77.3	-	-	76.8	-	74.7	-	-	75.8	-	-
C. Cons.	-	-	78.2	-	-	77.1	-	-	77.3	-	-	79.7	-	-	75.2	-	74.5	-	-
C. Dosie	-	-	79.3	-	78.9	-	-	79.3	-	-	80.0	-	-	76.3	-	-	73.3	-	-
W. Vani	-	-	77.6	-	-	79.3	-	-	79.6	-	75.6	-	-	-	78.0	-	77.3	-	-
Patsy	-	-	-	-	78.3	-	-	76.8	-	-	75.1	-	76.8	-	-	78.7	-	-	72.6
S. Adel	-	-	-	-	75.4	-	78.2	-	-	76.3	-	-	75.6	-	-	78.8	-	-	76.9
S. Greta	-	-	-	78.9	-	-	80.5	-	-	78.4	-	-	74.3	-	-	78.5	-	-	77.5
D. Mona	-	-	-	75.5	-	-	77.1	-	-	77.5	-	-	72.0	-	-	74.2	-	-	69.9

Showing the Casein number for the last 18 weeks before end of lactation.

TABLE B. II

SHOWING THE 95% PROBABILITY RANGE OF CASEIN NUMBER DURING THE LAST 18 WEEKS OF LACTATION

Week before end of Lactation	No. of Samples	Standard Deviation	$m - 2\sigma$	Mean	$m + 2\sigma$
18	3	.99	75.72	77.70	79.68
17	5	.79	76.72	78.30	79.88
16	6	2.37	72.36	77.10	81.84
15	9	2.70	73.5	78.90	84.3
14	6	1.61	75.78	79.0	82.22
13	8	2.26	72.48	77.0	81.52
12	7	2.06	72.78	76.9	81.02
11	5	1.17	74.86	77.2	79.54
10	10	2.03	72.54	76.6	80.66
9	7	1.80	72.8	76.4	80.00
8	6	2.53	69.94	75.0	80.06
7	7	1.55	71.3	74.4	77.5
6	8	2.36	70.38	75.1	79.82
5	6	2.22	70.46	75.9	80.34
4	8	2.54	71.02	76.1	81.18
3	9	2.51	69.08	74.1	79.12
2	2	3.47	65.36	72.3	79.24
1	11	4.13	63.44	71.7	79.96



GRAPH II.

Showing the variation of the casein number during the last 18 weeks of lactation.

SUMMARY.

(1) The effect of mastitis on milk constituents was investigated. The casein number was found to be lower in case of cows suffering from mastitis. It is suggested that indirect methods for diagnosis of mastitis should be applied side by side with the direct methods to overcome the difficulty caused by the intermittent excretion of the organisms.

(2) Acetonuria causes a reduction in the yield of milk, but it returns to normal after a short time.

(3) An abnormal condition which lowers the milk yield tends to increase the percentages of the milk constituents.

(4) Oestrum did not affect the yield of milk or the percentages of protein and casein.

(5) The casein number method of diagnosing mastitis cannot be applied towards the end of the lactation period.

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PART III.

THE RELATIONS BETWEEN MILK YIELD, BUTTERFAT AND PROTEIN.

THE RELATIONS BETWEEN MILK YIELD, BUTTERFAT AND PROTEIN.

The study of the relationships between milk yield and different milk constituents should throw light on the mechanism of milk secretion. It should provide a means of determining the probable behaviour of the various constituents, if breeding policy is directed towards the increase of the milk yield or of any given constituent.

The relation between the milk yield and butterfat was examined by Krizenecky (1933) who summarised the results of previous work and came to the conclusion that the variation of milk yield and of absolute fat yield is practically the same in each case, the average coefficient of variability being 1:1.037. He found high positive correlation between the milk yield and absolute fat yield, i.e. 0.8432. The coefficient of correlation between milk yield and fat percentage - when calculated for different groups - was negative and low, i.e. -.1988 to -.1842. He found that the negative correlation between milk yield and fat percentage is overpowered by the positive correlation between milk yield and fat yield, and consequently fat yield will follow an increase in milk yield.

Varecka (1935) confirmed Krizenecky's results. He found that the correlation coefficient between milk yield and butterfat yield is + .8824, and that between fat percentage and yield is -.1374. The coefficient of correlation between fat per cent and fat yield was low and positive, i.e. + .1741. He says that the limit of milk yield for the cow, an increase beyond which will lead to a decrease of fat production owing to a fall of fat percentage, lies between 17,603 and 39,189 kg. and is therefore entirely outside practical possibility.

Krizenecky and Lantuskin (1935) and Varecka (1935) found that the correlation between milk yield and butterfat yield is practically linear, at least within the limits of normal variation of milk yield. Krizenecky and Lantuskin (1935) state that "the curve for absolute fat production follows that for milk yield in a parabola".

Becker (1934) analysed milk from cows in the 4th, 5th and 6th week of lactation for the percentages of protein content and butterfat. Comparison of the means showed a slight positive correlation between the fat and protein percentages $r = .37$. He concludes that there is no need to fear that a rise in fat percentage will lead to a fall in protein content.

Azarme (1938) studied the correlations between the percentages and absolute amounts of different proteins and the milk yield. He found high positive correlations between milk yield and the yields of the different proteins, and a slight negative correlation between the percentages and the yield of milk.

Gaines and Overman (1938) investigated the inter-relations of milk-fat, milk-protein and milk energy yield. Their chemical analysis was carried out on 3-day samples at 5-week intervals. They found the coefficient of correlation between the percentage of fat and that of protein to be $r = +.755$.

Material and Methods.

Protein, casein, albumin + globulin and butter-fat percentages were determined in more than 1200 milk samples. Methods of determination for proteins were already mentioned in Part I. Fat percentage was determined by Gerbers' method in the morning and evening samples separately. The amount of protein secreted in 24 hours was obtained by multiplying the milk yield by the protein percentage found in the sample. The amount of butterfat was obtained by multiplying the morning and evening milk yields by the corresponding percentages of fat, and adding the two together. The percentage of butterfat was obtained by dividing the butterfat yield as above by the twenty four hours milk yield instead of by averaging the two butterfat percentages, since the morning and evening milk yields are not identical, i.e. morning milk is usually higher in amount and lower in its percentage of fat than the evening yield.

Statistical Analysis of the data.

The object of this paper is to investigate the correlation between butterfat and protein for a constant volume of milk. This can be done in two ways. For the first method the following three coefficients of correlation are required:-

Section of the thesis

(a) between milk yield and butterfat yield;

(b) between milk yield and total protein nitrogen;

(c) between butterfat yield and total protein nitrogen.

From these three figures it is possible to obtain a fourth, which denotes the partial correlation between the yields of protein and butterfat for a constant milk yield.

The second method is by correlating the percentages of protein and butterfat in each sample, and the values so obtained are graphically represented, as shown in Table IV.

Both methods were employed. Table I shows the correlation surface for the amount of protein and the milk yield. To arrive at an accurate result, it is necessary to ensure that the classes should be less than a quarter of the standard deviation (Fisher 1936). The correlation between milk yield and amount of protein was found to be high and positive.

$$r = +.939 \pm .0022 \qquad (1)$$

The correlation coefficient between amount of butterfat and milk yield was found to be :-

$$r = +.932 \pm .0022 \qquad (2)$$

Table II shows the correlation surface for butterfat and milk yield.

Finally, the coefficient of correlation between the yield of butterfat and protein was found to be:-

$$r = +.928 \pm .0027 \qquad (3)$$

Table III. shows the correlation surface for the amounts of butterfat and protein.

Using Fisher's formula (1936, p.190)

$$r_{12.3} = \frac{r_{12} - r_{13}\ r_{23}}{(1 - r_{13}^{2})(1 - r_{23}^{2})}.$$

when $r_{12.3}$ is the correlation between butterfat and protein for a constant milk yield

r_{12}, r_{13} and r_{23} are the correlation coefficients between butterfat and protein, protein and milk yield and between butterfat and milk yield respectively.

The partial correlation thus found was :-

$$r_{12.3} = +.429 \qquad (4)$$

When the second method (by correlating the two percentages) was adopted, the coefficient of correlation was found to be :-

$$r = +.343 \pm .0173 \qquad (5)$$

Table IV shows the correlation surface for the percentage of butterfat and percentage of protein.

DISCUSSION AND CONCLUSION.

Becker (1934) analysed milk from cows in the 4th, 5th and 6th week of lactation for the percentages of protein content and butterfat. After comparing the means he found a slight positive correlation between the fat and protein percentages $r = +.37$. He concluded that there is no need to fear that a rise in fat percentage will lead to a fall in protein content. The writer agrees with him about the absolute figure for the coefficient of correlation and objects to the way in which the conclusion was derived. The present results are in agreement with Becker's, in that there is a slight positive correlation between the two percentages. It was shown (Azarme, 1938; Krizenecky, 1933, 1934; and Varecka, 1935) that both the percentages are negatively correlated with milk yield, and that the amount of both butterfat and protein are highly positively correlated with the milk yield.

It may be concluded that if the fat percentage rises it will be accompanied by a rise in the protein percentage and a fall in the milk yield, as well as in the amounts of fat and protein secreted. The fall in the amount of protein should be more than that added by the rise in the protein percentage, since the correlation between the milk yield and protein amount is higher than that between the milk yield and protein percentage.

The author concludes from the present figures (correlations between yields of milk, butterfat and protein) that there is no fear, in pursuing a policy for increasing milk production, of decreasing the total yields of protein or butterfat. The result of such a policy will be cows giving high yields of milk, butterfat and protein, but this milk will possess lower percentages of butterfat and protein. Krizenecky's results (1933) are in full agreement with this statement as regards butterfat.

Gaines and Overman (1938) found that the coefficient of correlation between the percentage butterfat and that of protein was $r = +.755$. The present results, however, do not agree with this figure. By using two different methods in the statistical analysis the coefficient of correlation was found to be + .429 and +.343 (i.e. denoting a moderate degree of positive correlation).

The coefficient of correlation between the two amounts of protein and butterfat being +.928 and the partial correlation being +.429 shows that we must not depend upon a coefficient of correlation between two variates if each of them is simultaneously correlated with a third.

SUMMARY.

The coefficient of correlation between 24 hours protein yield and that of butterfat was found to be high and positive (+.928 ± .0027). Similarly, high values were found for the amount of butterfat and milk yield (+0.932 ± .0022), and for the amount of protein and milk yield (+.939 ± .0022).

The partial correlation between the amounts of protein and butterfat was found to be .429. However, when the two percentages were correlated the result was +.343 ± .0173. Both these denote a moderate degree of positive correlation between protein and butterfat for a constant volume of milk.

Pursuing a policy for increased milk production results in a high milk yield with increased net amounts of protein and butterfat, although the percentage content of these constituents is somewhat diminished.

TABLE I.

SHOWING THE CORRELATION SURFACE FOR THE AMOUNT OF PROTEIN AND MILK YIELD.

AMOUNT OF PROTEIN NITROGEN (1/1000th lb. per 24 hours).

MILK YIELD (lb. per 24 hours).	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	TOTAL
6		4	8	1																															13
9			10	13	3																														26
12				5	30	20	2																												57
15					2	30	26	2	1																										61
18						5	14	29	20	2																									70
21							1	8	44	24	3																								80
24									9	26	51	13	5	1				1																	106
27									2	1	25	52	13	7																					100
30											7	16	34	44	6	3	1																		111
33												3	17	37	45	13	4	1	1	1															122
36													1	11	28	30	21	7	1	1	1														101
39														1	8	24	28	12	7	3	1														83
42															2	8	14	27	15	8		2	1												77
45															1	1	3	14	14	12	7	3	1	1											57
48																		3	9	17	14	6	6												55
51																		2	1	13	10	2	6	3					1						38
54																				1	5	9	6	5	3										29
57																				3	1	7	5	7	5	2	1	1							32
60																					1	1	3	3	9	7	4								28
63																								3	3	4	4	2	2						18
66																									1			1	1	1	2				6
69																											1			2					3
72																																		1	1
75																															1				1
TOTAL		4	18	19	35	55	43	39	76	53	86	84	70	101	90	79	71	67	48	59	40	30	28	22	21	13	10	4	4	3	3			1	1276

TABLE II

SHOWING THE CORRELATION SURFACE FOR THE AMOUNT OF BUTTER-FAT AND MILK YIELD.

AMOUNT OF BUTTERFAT (1/100th lb. per 24 hours).

MILK YIELD (lb. per 24 hours).	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	TOTAL
3																																
6		4	9																													13
9		1	10	10	1																											22
12			3	15	25	10	2	1	1																							57
15				2	15	29	10	2	2	1																						61
18				1	1	13	26	21	4	3																						69
21				1		3	13	33	16	6	3	2																				77
24						3	2	22	35	29	6	5			1																	103
27					1	1		4	21	32	21	11	3		1			1	1													97
30							1	1	8	24	24	23	15	7	2	3																108
33								3	2	9	16	40	29	10	5	4	1															119
36										4	8	18	23	22	12	9	1	1	1	1				1								101
39											2	10	20	19	14	11	4		1		1											82
42											1	5	4	12	14	23	9	4	3	1												76
45													3	6	7	19	5	9	3	2												54
48									1			1		3	2	7	18	6	6	4	3	1				1						53
51													2		4	9	4	1	4	4	6		2							1		37
54															1	1	4	6	3	1	4	2	4	2								28
57																2		4	4	6	5	5		2	1							29
60																	1	2	2	2	7	7	4	2	1	1						29
63																		1		4	2	2	2	3	2	1	1					18
66																	1				1	1		1	2							6
69																						1	2									3
72																								1								1
75																								1								1
Total		5	22	29	43	59	54	87	90	108	81	115	99	79	63	88	48	35	28	25	29	19	14	13	6	3	1			1		1244

TABLE III

SHOWING THE CORRELATION SURFACE FOR THE AMOUNT OF PROTEIN AND BUTTERFAT.

AMOUNT OF TOTAL PROTEIN (1/1000th lb. per 24 hours).

AMOUNT OF BUTTERFAT (1/100th lb. per 24 hours).

	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	250	260	270	280	290	300	310	320	330	340	350	TOTAL
20		2	3																																5
30		2	12	5	3																														22
40			2	9	11	5	1		1																										29
50				3	17	20	2	1				1																							44
60					4	20	20	7	3	1	2	1																							58
70						4	15	17	12	4		1	1	1																					55
80						2	3	14	30	19	10	5	1		2																				86
90						2	2		13	13	34	12	8	4	1					1															90
100						1			8	10	22	29	13	16	5	3	1																		108
110									1	6	6	19	17	12	10	4	4	1																	80
120								1			6	7	17	35	20	15	7	3	5	1															117
130												5	3	20	25	16	15	8	2	3	2														99
140												2	4	2	12	17	20	9	6	5	1														78
150									1			1	1	2	6	11	8	18	5	6	2	1	1												63
160											2		2	3	5	5	10	15	15	15	7	3	4	1	2										89
170														1	4	1	2	5	7	11	9	2	1	2											45
180											1		1			1	1	5	2	5	3	5	5	3	1	2	1								36
190																1	2	1	1	5	4	5	3	2	1	2									27
200																		1	1	2	5	3	3	3	4		3								25
210																1				1	6	4	5	4	4	1		1	1						28
220																					1	2	1	1	6	3	2	2							18
230																					1	2		4	1	2			2	2					14
240															1							1	1	1		2	2		1		2			1	12
250																						1			1		1	1		1					5
260																							1	1		1									3
270																											1								1
280																																			
290																																			
300																				1															1
310																																			
Total		4	17	17	35	54	43	40	69	53	83	83	68	96	91	75	70	66	44	56	41	29	25	22	20	13	10	4	4	3	2			1	1238

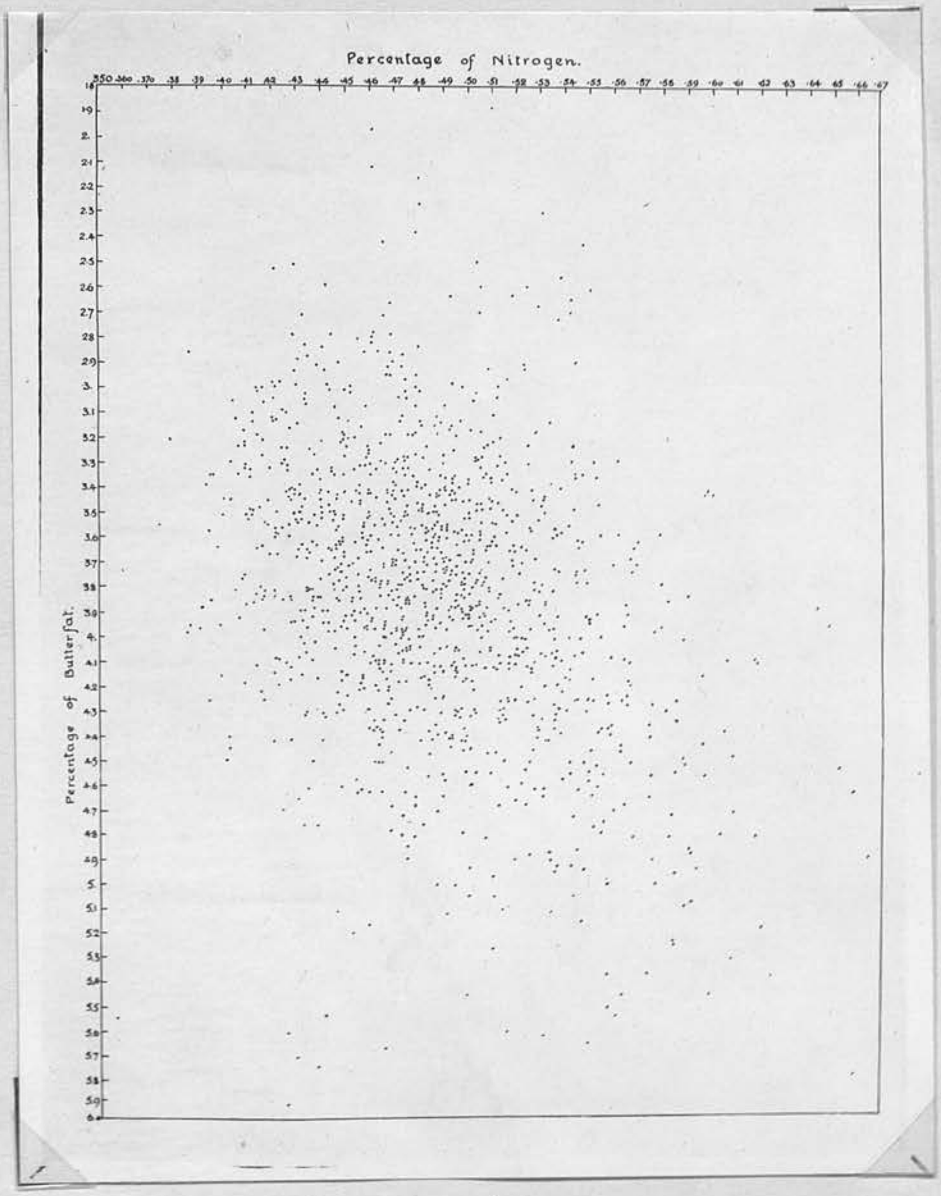

Table IV

Showing the correlation surface for percent butterfat and percent protein nitrogen.

Samples containing the following protein nitrogen and fat percentages are not included in the correlation surface although they are taken into consideration while calculating the coefficient of correlation between the two percentages.

Protein Nitrogen %	Fat %
.445	6.60
.559	6.17
.530	6.17
.543	6.22
.650	6.06
.830	5.11
.787	4.13
.823	5.27
.435	6.42
.689	4.00
.801	4.42
.626	6.06
.684	4.00
.729	5.14
.676	4.31
.539	6.08
.681	4.36
.507	6.88
.536	7.10
.584	7.90

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